THE PHOSPHORUS NUTRITION OF TWO GRAIN LEGUMES AS AFFECTED BY MODE OF NITROGEN NUTRITION

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ABSTRACT

Two sand culture experiments were conducted to examine the effects of P stress, nodulation, and N source on the growth, dry matter distribution (DMD), and root development of soybean (Glycine max (L.) Merr.). In both experiments two levels of nitrogen (0 and 5.0 mM N) were employed to establish two contrasting modes of N nutrition: plants were either (1) solely dependent upon symbiotic N fixation, or (2) primarily dependent upon uptake of combined N from the nutrient solution. Total dry weight of N-fixing plants grown at optimal P levels was approximately 60% that of N-supplied plants. Mode of N nutrition had no effect upon the total dry weight of plants grown at deficient P levels. The DMD within the plant differed depending upon the P and N supply. Nodule dry weight of N-fixing plants grown at optimal P levels comprised 9% of the total plant dry weight and 61% of the root dry weight of 35 day old soybean. A decrease in the P supply inhibited nodule growth relatively more than either root or shoot growth. Nodule dry weight of N-supplied plants grown at optimal P levels comprised 2% of total plant weight and 15% of root dry weight and a decrease in the P supply affected shoot growth relatively more than either nodule or root growth.

The higher yields of N-supplied plants resulted from increased early growth during the time when the plants not provided combined N were establishing an N-fixing, root-nodule system.

When grown at suboptimal P levels, there was a similar partitioning of dry matter between the underground portion of the plant and the shoot in both N-fixing and N-supplied plants. However, the root:total plant dry weight ratio of N-fixing plants was significantly less than that of

N-supplied plants. This difference was attributed to the larger nodule mass on N-fixing plants. There was an inverse relationship between nodule mass and total root length although the number of first-order lateral roots on nodulated and nonnodulated plants was the same.

The data suggest that two functional equilibriums are operative in the N-fixing plant, namely, the partitioning of dry matter between (1) the underground portion of the plant and the shoot and, (2) the root and nodules. Phosphorus stress affected the root-nodule equilibrium relatively more than the partitioning of dry matter between the below-ground and above-ground parts of the plant. In N-supplied plants, P stress primarily affected the partitioning of dry matter between the root and shoot.

An N x P field experiment with a split-plot randomized block design was conducted to identify the critical external and internal P requirements of both soybean and cowpea (Vigna unguiculata (L.) Walp.) as affected by mode of nitrogen nutrition. Six P treatments were established in mainplots on a Humoxic Tropohumult soil. Within each mainplot an N-deficient and N-luxuriant subplot was established: sugarcane bagasse was incorporated into the entire field at a rate of 16,000 kg/ha; N-deficient subplots received no urea-N applications while N-luxuriant subplots received applications of urea-N before planting and during crop growth totaling 1000 kg/ha. In vitro monitoring of the net N mineralization of incubated soil samples collected from N-deficient subplots during crop growth indicated that the bagasse immobilized most of the available soil N during the first seven weeks of crop growth and that plants in these treatments were predominantly dependent upon symbiotic N fixation to meet

their N requirement for growth. Acetylene reduction activity and nodule dry weight of soybean and cowpea plants from N-luxuriant subplots sampled 32 and 46 days after emergence were less than 11% of their counterparts from N-deficient subplots grown at comparable P levels. This indicated that plants from the N-luxuriant treatments were primarily dependent upon uptake of combined N from the soil to meet their N requirement for growth.

Results from the field experiment showed that soybean was more sensitive to low soil P than was cowpea. When grown without P or N fertilizer, soybean yielded 28% of the maximum yield obtained from optimal P treatments on N-deficient subplots while the comparable relative yield for cowpea was 72%. The sensitivity of N-fixing soybean to low soil P levels when grown on N-deficient soil could be characterized by: (1) a relative growth rate (RGR) which declined progressively throughout the crop growth period, (2) lower index tissue and seed P concentrations than the N-supplied soybean plants grown at comparable P levels although the critical internal P concentration required for 90% maximum yield of N-fixing and N-supplied plants was the same, (3) an external P requirement approximately 60% higher than N-supplied soybean plants and, (4) a relatively larger difference between the yield potentials of N-fixing and N-supplied plants than for cowpea. The tolerance of N-fixing cowpea to low soil P when grown on N-deficient soil could be characterized by: (1) a high RGR during the later stages of growth, (2) tissue P concentrations which were similar to those of N-supplied cowpea plants grown at comparable P-levels, (3) an external P requirement which was not affected by soil N availability and, (4) a relatively smaller difference between the yield potentials of N-fixing and N-supplied plants than for soybean.

It was concluded that screening of grain legumes for tolerance to low soil fertility levels should be conducted on N-deficient soils to insure that nutrient requirements are assessed for the symbiotic, N-fixing plant.

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CHAPTER I

THE PHOSPHORUS NUTRITION OF TWO GRAIN LEGUMES AS AFFECTED BY THE MODE OF NITROGEN NUTRITION

INTRODUCTION

On planet earth there exists a tenuous balance between human food consumption and human food production. In the nine year period from 1961 to 1970, the number of Homo sapiens in the world increased at an average annual rate of 2.0% while worldwide food production increased at an average annual rate of 2.5% (Sanchez, 1976). Most of the increased food production occurred in developed countries. In Africa, for example, population growth was greater than the increase in food production during the same nine year period.

Kellogg and Orvedal (1969) stated that most of the potentially arable but presently unused soils of the world are in the tropics, a region which includes most of the developing countries of the world. A tropical soils map compiled by Aubert and Tavernier (1972) indicated that Oxisols and Ultisols occupy 22% and 11%, respectively, of the soils in the tropics. Kellogg and Ordeval (1969) concluded that one of the major factors limiting the utilization of tropical areas suited for crop production is inadequate knowledge of how to manage the highly weathered Oxisols and Ultisols in these regions. Uehara (1977) estimates that approximately 500 million hectares of potentially arable land occur in the humid and semi-arid tropics. Although low temperatures, light, and water would not prohibit the development of these regions, the high cost of soil amendments required to bring these lands into agronomic production does pose a severe economic restraint. Nitrogen, phosphorus and lime

are the three major fertilizer inputs which are needed on these soils.

Grain legumes are the only major human food crops which symbiotically fix substantial quantities of atmospheric N_2 during crop growth. They can provide high protein food without the use of industrially produced N fertilizers. However, the nutritional requirements of symbiotic, N-fixing legumes differ from their N-supplied counterparts (Munns, 1977). Thus it is apparent that utilization of grain legumes in cropping systems on the potentially arable soils of the tropics will necessitate research which elucidates the specific nutritional requirements of symbiotic, N-fixing grain legumes.

The experiments conducted by the author and discussed in this dissertation were designed to address two questions: (1) What are the external soil and internal tissue P requirements of effectively nodulated soybean (Glycine max (L.) Merr.) and cowpea (Vigna unguiculata (L.) Walp.) when grown on an N-deficient soil? and, (2) Are the P requirements of these two grain legumes affected by the supply of available soil N? Three studies were conducted to answer these questions, each described and discussed in a separate chapter in this dissertation. The first study involved a greenhouse sand culture experiments with soybean which were designed to examine the effects of P stress, nodulation, and mode of N nutrition on the growth and partitioning of dry matter within the plant. The results are presented in Chapter III. A second study (Chapter IV) examined the relationship between soil nitrogen availability and the predominant mode of N nutrition during crop growth for both soybean and cowpea. A field

experiment which was designed to compare the P nutrition of symbiotic, N-fixing and N-supplied grain legumes is discussed in Chapter V. The general conclusions from these investigations are discussed in the final chapter.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

There are two possible modes of nitrogen nutrition for legumes which have the potential to form symbiotic associations with Rhizobium: (1) the legume may absorb N that is available in the soil and (2) the rhizobial microsymbiont may reduce atmospheric N_2 in the root-nodule which is subsequently assimilated by the plant. These two distinct modes of N nutrition are illustrated in Figure 1. Although an effectively nodulated legume is considered to be taxonomically identical to its nonnodulated counterpart, the morphology and physiology of their roots differ markedly. The effect of the mode of N nutrition on the nutritional requirements of legumes is the subject of this literature review. However, an adequate understanding of the requirements of N-fixing and N-supplied legumes (with special emphasis on P nutrition), requires a review of the literature concerning legume physiology, soil N availability, soil P availability, and enhanced P uptake by mycorrhizal root infection.

Morphological and physiological differences.

The most obvious difference between N-supplied and N-fixing legumes is the presence of nodule structures on the roots of N-fixing plants. The nodule dry weight of effectively nodulated legumes may comprise a significant proportion of the total plant biomass. Summerfield et al (1977) reported that nodules on 46 day old cowpea plants comprise

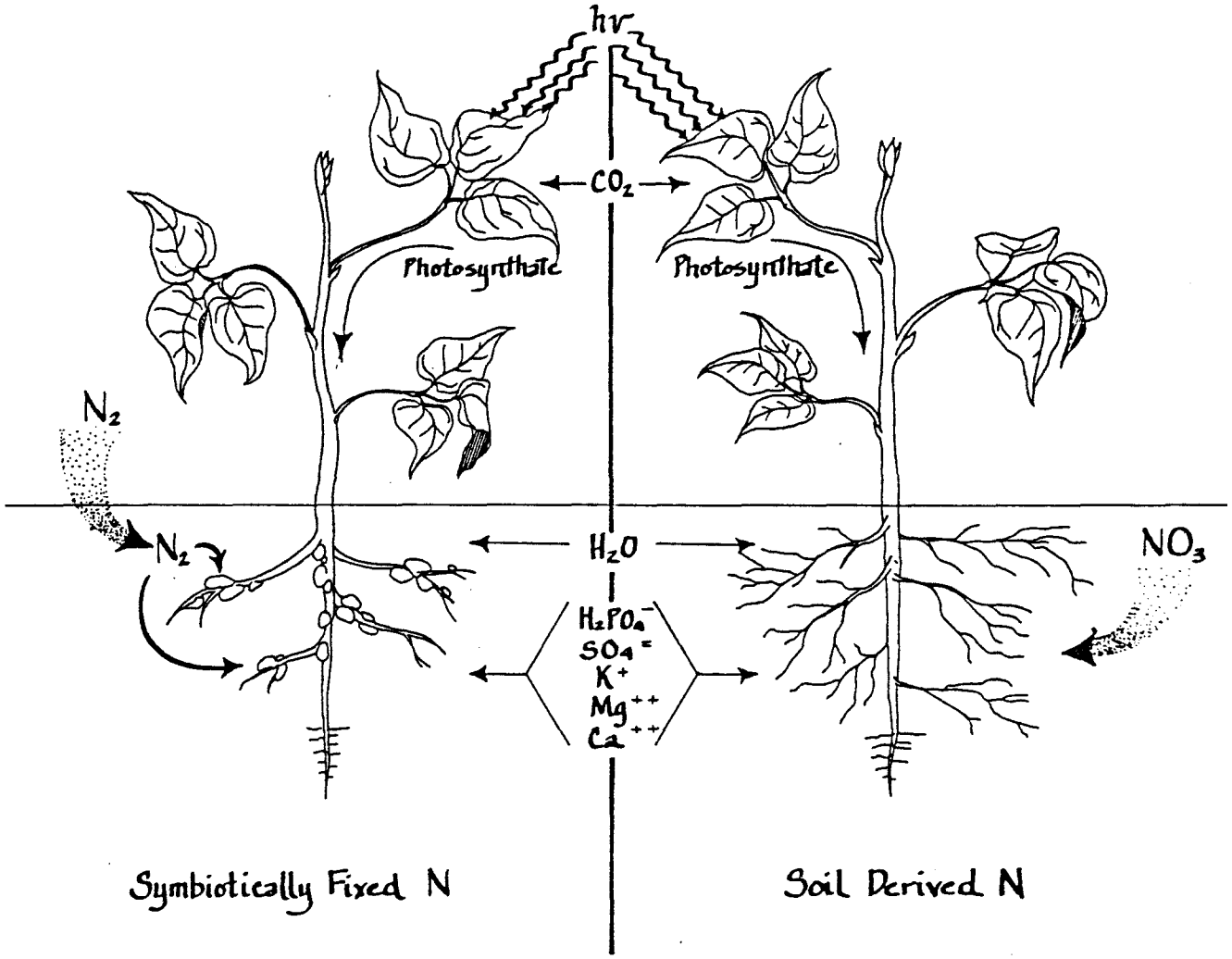


Figure 1. Two modes of nitrogen nutrition by legumes.

4% of the total plant dry weight and 32% of the root dry weight. Wych and Rains (1978) found the nodules of 32 day old soybean plants comprised 6% of the total plant weight and 20% of the root weight. By direct measurement of photosynthesis, carbon accumulation in dry matter, respiratory output of plant organs, and organic solute exchange between root and shoot, Minchin and Pate (1973) showed that the root-nodules of pea (Pisum sativum L.) utilized 32% of the net gained photosynthate during a nine day period from 21 to 30 days after planting. The comparable figure for cowpea is 14% for the 0 to 61 day period following planting (Herridge and Pate, 1973). A nonnodulated legume does not have this additional photosynthate sink although it does expend energy reducing nitrate when NO_3-N is the source of available N.

Root morphology of nodulated and nonnodulated legumes have also been shown to differ. Physiological studies of red clover (Trifolium pratense L.) nodulation led Nutman to hypothesize that nodule infection occurs at lateral root initiation sites. Dart and Pate (1959) reported a similar relationship in their studies with barrel medic (Medicago tribuloides L.). In experiments with soybean, Wych and Rains (1978) and Weber (1966) noted that root systems of N-supplied plants were more fibrous than were those of N-fixing plants. Puppo and Rigaud (1978) found a higher cytokinin activity in -N nutrient solutions in which nodulated French bean (Phaseolus vulgaris L.) had been grown than in comparable +N nutrient solutions in which nonnodulated plants had grown. In these same experiments they noted that the nodulated bean plants had fewer lateral roots than uninoculated plants. Cytokinins have been shown to inhibit lateral root development (Street, 1969). Broughton (in press)

found that root growth of inoculated soybean and cowpea was slow during early growth when the plants were establishing an effective symbiosis. Root growth of N-supplied plants was faster during this same period.

Specific enzymes and proteins that are required for symbiotic N fixation are not found in nonnodulated plants. The nitrogenase enzyme and leghaemoglobin are only found in effective root-nodules of N-fixing legumes (Virtanen, 1947; Evans and Russell, 1971). On the other hand, no nitrate reductase activity was found in the leaves of nodulated soybean plants grown in -N nutrient solution gravel culture while NO_3-N supplied plants had high nitrate reductase activity in their leaves (Harper, 1974).

The internal plant N pathways are different in N-fixing and N-supplied plants. Oghoghorie and Pate (1972) showed that most of the N assimilated by the roots of field peas (Pisum arvense L.) is cycled first through the shoot when plants are solely dependent on symbiotically-fixed N whereas N-supplied plant roots assimilated NO_3-N directly after uptake from the nutrient solution.

Thus, N-fixing and N-supplied legumes differ in their root morphology and structure, in plant dry matter distribution and partitioning of photosynthate among plant organs, and in their biochemistry. These are all good reasons to suspect that their nutrient requirements will differ also.

Nutritional requirements.

Nitrogen-fixing legumes appear to have greater requirements for some of the essential mineral nutrients than do their N-supplied

counterparts. Anderson and Spencer (1950) reported that N-fixing legumes had a higher Mo requirement than non-legumes grown on the same soil. Molybdenum is a co-factor in the nitrogenase enzyme and appears to be required for the reduction of atmospheric N_2 by the nodule bacteria (Evans and Russell, 1971). Although cobalt has been shown to be a required element for symbiotic legumes (Ahmed and Evans, 1960; Reisenauer, 1960), there is no satisfactory evidence that plants require Co for growth in the presence of N. Calcium also appears to be required in greater amounts by some N-fixing legumes (Loneragan, 1959; Lowther and Loneragan, 1970).

Munns (1977) stated that, "Deficiencies of phosphorus, sulfur, and potassium severely and frequently limit N_2 fixation by limiting the growth of the host plant. Although there are no clear demonstrations that they directly limit nodulation or N_2 fixation, there are data suggestive of such effects in soil experiments." This is still the present state-of-the-art. Some studies have shown that applications of P or K fertilizers to soil increased the N content of legumes growing on those soils (McLachlan and Norman, 1961; Albrecht et al, 1940). This has led many agronomists to conclude that P and K act directly to increase N fixation. However, these experiments did not include the N control treatments necessary to confirm these hypotheses.

Effect of combined N.

In the landmark work of Allos and Bartholomew (1959), the effect of combined N on symbiotic N fixation in five legume species was similar in that: (1) the symbiosis never supplied sufficient N for

maximum growth and, (2) application of combined N in quantities greater than that needed for maximum growth served to inhibit N fixation completely. The second observation suggests that legumes preferentially assimilate combined N when sufficient quantities are available. The first observation implies that there is some "cost" in terms of yield resulting from total dependence on symbiotic N fixation. More recent studies have shown that large applications of combined N to an inoculated legume inhibit nodulation (Munns, 1968), reduce nodule development, and decrease N fixation (Weber, 1966). However, results from field experiments conducted to compare the yield potential of symbiotic, N-fixing versus N-supplied legumes are inconsistent. Weber (1966) reported that yields from nonnodulating soybean supplied 670 kg N/ha were equivalent to the yield from effectively nodulated soybeans which were not N-fertilized when both crops were grown on soil in which 45,000 kg/ha of ground corncobs had been incorporated. Although the amount of N applied in that experiment would appear to be extravagent for normal soil conditions, it is impossible to estimate how much of the applied N remained available to the plant. No direct measurements of soil N availability were made in that study. Others have also found no yield increase from applied N in field experiments with soybean (Pal and Saxena, 1975; Welch et al, 1973; Olsen et al, 1975). However, the field experiments conducted by Bhangoo and Albritton (1976), Norman (1943), and Kang (1975) all showed that N-fertilized soybean yielded significantly more than inoculated plants which did not receive N fertilizer. A reasonable hypothesis is that the inconsistent response to N fertilizer is a result of differences in soil N availability in these experiments. In sand, gravel, or

nutrient solution culture systems the response to combined N is more consistent (Allos and Bartholomew, 1959; Summerfield et al, 1977; Latimore et al, 1977; Ryle et al, 1978; Wych and Rains, 1978).

Soil N availability.

Soil N relations are extremely complex. The availability of N in soil varies with soil organic matter content, temperature, water content, depth, and aeration. However, recent studies have shown that there are certain consistencies among soils in their N mineralization rates. The N mineralization rates in most soils: (1) are proportional to the total amount of mineralizable N present in the soil (Stanford and Smith, 1972), (2) are similarly influenced by changes in temperature, most having a Q_{10} of 2 (Stanford et al, 1973), (3) are optimal at approximately the moisture content equivalent to field capacity (Stanford and Epstein, 1974) and, (4) are reasonable estimates of actual soil N availability as estimated by N uptake by plants (Stanford et al, 1973). These similarities among soils indicate that direct measurement of N mineralization during crop growth may clarify some of the contradictory reports concerning the effect of combined N on the yield potential of N-fixing legumes.

Soil P and P uptake by plants.

The concentration of P in most soil solutions is extremely low, usually much lower than the concentrations of other essential plant macronutrients (Reisenauer, 1966). As a result, the supply of P available to the plant is dependent upon its diffusion to the root.

Diffusion of P in the soil solution to the root is described by the equation:

$$D_p = D_e \theta f_1 \gamma (dC_1/dC)$$

where D_e is the diffusion coefficient of $PO_4^{=}$ in water, θ is the volumetric moisture content, f_1 is the tortuosity factor, γ is a factor accounting for negative adsorption and water viscosity effects, and dC_1/dC accounts for the buffering capacity of the soil (Barber, 1974).

Many tropical soils are highly weathered and are composed of oxides and hydrous oxides of aluminum and iron. In these soils adsorption of phosphate to the colloidal surfaces is generally strong and the total P adsorption capacity of the soil is relatively high (Fox and Searle, 1978). Phosphorus sorption makes it difficult to assess the availability of P to plants in highly weathered soils. Fox and Kamprath (1970) proposed using P sorption isotherms as a basis for evaluating the P availability in soil. This method provides estimates of two important parameters, namely, the buffering capacity of the soil and the concentration of P in the soil solution. In subsequent studies, this method has proven to be useful in predicting the external P requirements of many crops on soils of diverse origins (Nishimoto et al, 1977; Peaslee and Fox, 1978; Howeler and Leon, 1977). The values obtained by this method are close to those obtained by Asher and Loneragan (1967) for a number of plants grown in a large volume, constant flowing solution culture system with continual P replenishment.

Soluble P added to soil generally reacts quickly with soil at first although the P in the soil solution declines slowly over a long period of time (Munns and Fox, 1976). This slow reaction makes it

difficult to compare the P requirements of different soils which had been previously P fertilized. Characterization of the slow reaction on different soils and the subsequent effect upon the predicted plant P requirement as estimated by the P sorption isotherm method warrants further study.

Soil N availability and form have also been shown to affect P uptake by crops. Nitrogen fertilizer applied with P fertilizer stimulates P uptake by many crops. Englestad and Allen (1971) found that the N x P interaction was greatest when the N was supplied as NH_4-N and banded with the applied P fertilizer. In the same study, NO_3-N did not increase P uptake regardless of placement. Leonce and Miller (1966) also found P uptake by corn to be increased by NH_4-N and not with NO_3-N. Riley and Barber (1971) showed that increased P uptake by soybeans supplied with NH_4-N was caused by a lowering of the pH in the rhizocylinder soil. They postulated that to maintain neutrality in the soil solution, the root exchanges H^+ ions for NH_4^+ in the absorption process. The resulting lowered pH around the root appears to increase P availability by conversion of $HPO_4^=$ to $H_2PO_4^-$ which is a more soluble form of phosphate. Thus, this evidence suggests that the N x P interaction will be minimized when: (1) NO_3-N is the most prevalent form of N in the soil, (2) the P is well mixed in the entire soil volume and fully equilibrated with the soil collodial surfaces, and (3) the soil has a high pH buffering capacity.

Mycorrhizal infection of plant roots also increased P uptake, especially on P-deficient soils. Mycorrhiza appear to be ubiquitous. Possingham et al (1971) found all tropical legumes examined to be

infected by mycorrhiza. Hayman and Mosse (1971) reported a 12-fold increase in the yield of onions when inoculated with mycorrhiza and grown in a P-deficient, sterilized soil. Subsequent studies using ^{32}P showed that these yield increases were due to increased P uptake by the mycorrhizal plants (Hayman and Mosse, 1972). Although mycorrhizal root infection may decrease the external P requirement of a plant (Mosse, 1973), Hayman and Mosse (1972) demonstrated that mycorrhiza do not tap sources of soil P that are unavailable to the uninfected plant but rather mycorrhiza increase the effective absorption surface of the root by hyphal extension beyond the zone of depletion around the root.

Smith and Daft (1977) and Mosse et al (1976) have shown that mycorrhizal infection of nodulated legumes increased both N fixation and nodule weight when grown on a P-deficient, sterile soil. Hayman (1975) reported that the numbers of Endogone spores and mycorrhizal plant root infection decreased in plots that were N fertilized for many years. These results suggest that there may be an interaction between soil N availability and mycorrhizal populations in soils.

CONCLUSIONS

That the physiology and morphology of an effectively nodulated legume differs markedly from its nonnodulated, N-supplied counterpart is quite clear and their nutritional requirements for a few of the essential mineral nutrients have been shown to differ also. However, definitive field experiments comparing their requirements for other

plant nutrients have not been undertaken. Such studies are inherently complex due to the heterogeneity of soil N relations in most soils. Comparing the P nutrition of N-fixing and N-supplied legumes is further complicated by the reactivity of P with many tropical soils and the involvement of mycorrhiza in the P nutrition of most plants. Thus, to conduct a successful experiment of this type will require a definitive study of the effects of P stress on the nodulation, N fixation, and growth of N-supplied and N-fixing legumes (Chapter III), a complete characterization and modification of soil N availability (Chapter IV), and a careful assessment of soil P availability, mycorrhizal root infection, and legume growth and yield at different soil P and N levels (Chapter V). This is an extremely intricate and uniquely challenging research problem.

CHAPTER III

DRY MATTER DISTRIBUTION OF SOYBEAN AS AFFECTED BY PHOSPHORUS STRESS, NODULATION, AND NITROGEN SOURCE

INTRODUCTION

Grain legumes when grown in conjunction with their rhizobial microsymbiont are the only human food crops that assimilate significant quantities of atmospheric N_2 during crop growth. However, successful symbiotic N fixation by legumes requires substantial changes in root morphology and physiology. The root-nodules represent an added sink for photosynthate and mineral nutrients. As a sink, the nodules must compete with other plant organs for the assimilates they require. A nonnodulated legume does not have this additional assimilate sink although it does expend energy reducing nitrate when NO_3-N is the source of available N. Thus, for legumes which have the potential to form symbiotic associations with Rhizobium, the pattern of dry matter distribution (DMD) within the plant will differ depending upon its mode of N nutrition.

The partitioning of dry matter between root and shoot is a heritable characteristic determined by the genotype of the plant (Andrews, 1939; Shank, 1943). Root morphology likewise is considered to be genetically determined (Smith, 1934; Zobel, 1975; Street, 1969). For any given plant, the expression of these characteristics can be altered by environmental conditions. Deficiencies of essential mineral nutrients have been shown to affect both the DMD within the plant and lateral root development. Plants stressed for N or P tend to accumulate relatively more dry matter in their roots than do plants which

are adequately supplied (Turner, 1922; Brouwer, 1962). Weisum (1958) demonstrated that root branching in pea (Pisum sativum) was stimulated by nutrients as follows: NO_3-N>P≫K>Mg>Ca. Nitrate applied to a discrete root segment increased both the rate of lateral root extension and the number of lateral roots per unit length of root (Hackett, 1972; McIntyre and Raju, 1967; Drew, 1975).

The establishment of an active N-fixing nodule system on the roots of a legume complicates these relationships. During the vegetative stage of growth, root-nodules on plants which rely upon symbiotically fixed N utilize significant quantities of photosynthate for nodule growth and for the biochemical processes associated with N fixation (Minchin and Pate, 1972; Herridge and Page, 1977). Summerfield et al (1977) found that the root:shoot dry weight ratio in cowpea was larger in nonnodulated plants than in nodulated plants grown at equivalent levels of applied N. Experiments with red clover and barrel medic indicate that there is an inverse relationship between nodule number and lateral root formation (Nutman, 1948; Dart and Pate, 1959). Also, there are numerous qualitative observations concerning the differences between the root morphology of grain legumes provided combined N and those which are effectively nodulated and mostly dependent upon symbiotically fixed N (Weber, 1966; Wych and Rains, 1978).

The purpose of this study was to define the changes in DMD and root development in the soybean plant as influenced by the mode of N nutrition, by the magnitude of root nodulation, and by P stress.

MATERIALS AND METHODS

Plant culture technique.

Two pot experiments were conducted at the NifTAL Project facilities, island of Maui, Hawaii. In both experiments 3.8 liter pots were filled with 4 kg of silica sand which had been thoroughly rinsed with tap water to remove fine particles and then leached with two pot-volumes of demineralized water. Nutrient solutions were prepared with demineralized water. A basal nutrient solution was used containing 1.5 mM $CaCl_2$, 0.75 mM $MgSO_4$, 0.5 mM K_2SO_4, 10 µM H_3BO_3, 4 µM $FeSO_4$, 2 µM $MnSO_4$, 1 µM KI, 0.1 µM $NaMoO_4$, and 0.02 µM $CoCl_2$. In Experiment 1 the nutrient solution contained 0.5 mM KH_2PO_4. In Experiment 2 the P levels varied as will be described later. In both experiments the N concentration in the +N nutrient solutions was 5.0 mM supplied as 1.5 mM $Ca(NO_3)_2$ and 1.0 mM NH_4NO_3. The varying anion was Cl^- and the +N and -N nutrient solutions were the same with respect to all other ions. The pH was initially adjusted to 6.8 with 0.1 N NaOH.

Seeds of "Clark 63" soybean were surface sterilized, pre-germinated, and four seedlings planted in each pot. At planting each seedling was inoculated with 2 ml of a broth inoculum containing Rhizobium strain TAL 379 (=USDA 136 b = CB1809). Viable plate counts indicated that each seedling received $4x10^7$ viable rhizobia. One week after planting pots were thinned to the two most vigorous plants.

Experiment 1 employed two levels of N (0 and 5.0 mM N, hereafter designated -N and +N) and two inoculation treatments (no inoculum and inoculated, hereafter designated -I and +I) in a factorial design with

3 replications. Beginning three days after planting and continuing until the end of the experiment, pots were watered daily with 800 ml of the appropriate nutrient solutions. This was equivalent to the maximum water holding capacity of the 4 kg of silica sand in each pot. Excess nutrient solution was discarded in the drainage.

In Experiment 2 there were 6 P treatment levels (0.005, 0.02, 0.05, 0.20, 0.50, and 2.0 ppm P, hereafter designated P_1 through P_6) which were combined factorially with two N treatment levels (0 and 5.0 mM N, hereafter designated -N and +N). Each treatment was replicated 3 times. At planting, all pre-germinated seeds were inoculated as previously described. Starting 3 days after planting, pots were watered daily with 800 ml of nutrient solution containing the appropriate P concentration. The nitrogen regimes for all +N treatments were initiated 16 days after planting at which time all pots were fitted with a 5 liter nutrient solution reservoir. An aquarium pump was used to recycle the nutrient solution through each pot at a rate of 12 ml/minute, sufficient to cycle the entire nutrient solution through each pot thrice daily. The P concentration in the nutrient solution in each pot unit was monitored and replenished on a daily basis: 20 ml aliquots were collected from each pot and replicate samples pooled; the P concentration was measured using the method of Watanabe and Olsen (1965); P levels were then restored to the original treatment levels. The pH of the nutrient solution was also monitored and adjusted on a daily basis using 0.2 N KOH. Transpirational water loss was replenished with demineralized water. The entire nutrient solution in each pot unit was replaced with fresh solutions 23, 29, and 34 days after planting. The depletion of NO_3-N

from the +N treatments was determined for the 24 hour period following the final nutrient solution change (day 34) using the cadmium reduction method (APHA, 1975).

Growth Analysis and Acetylene Reduction Measurement.

In Experiment 1 plants were harvested 21 days after planting. Stems were cut at the sand surface, the roots carefully washed free of sand, and nodules picked from the fresh root samples and counted. The number of all first-order lateral roots on the tap root were counted. Lateral roots were then cut from the tap root and the total length of first and second-order lateral roots was measured directly. Roots, nodules, and shoots were dried at 55°C and weighed.

In Experiment 2 the treatments were harvested 35 days after planting between 1200 and 1400 hours. Stems were cut at the sand surface and roots gently removed from the sand. Root systems from each pot were immediately placed in 2 liter bottles, injected with 100 ml acetylene (0.05 atmosphere C_2H_2), and incubated for 1.5 hours. Ethylene was determined by gas chromatography. Nodules were picked from the root systems. The roots, shoots, and nodules were dried at 55°C and weighed.

RESULTS

Experiment 1.

The pre-germinated seedlings emerged from the sand 1 to 2 days after planting. When pots were thinned, 7 days after emergence, small nodules were visible on the roots of plants in the -N+I treatments.

No nodules were apparent on the roots of plants from -I or +N treatments. By day 14, during expansion of the first trifolioate leaf, the leaves on -N plants had become chlorotic. In the -N-I treatment, the plants became increasingly chlorotic until harvest. At day 16 the leaf-veins on plants in the -N+I treatment began to turn green and by harvest these plants were a normal color. At harvest +N plants were markedly larger and had a darker green leaf color than did -N+I plants.

The data from Experiment 1 is presented in Table 1. A few small nodules were found on the roots of some uninoculated plants. These nodules were immature and contained no visible leghaemoglobin which indicated that contamination occurred late in the experiment. Roots from the -N+I treatment were well nodulated; the largest nodules were observed on the upper portion of the tap root. The roots of plants from the +N+I treatment had numerous small nodules dispersed throughout the root system, most of which were too small to remove from the root for dry weight determination. Plants from +N+I treatments had more nodules but less total nodule dry weight than their -N+I counterparts.

Inoculated plants had less extensive root systems than uninoculated plants grown under the same N regime (Table 1). There was an inverse relationship between nodule dry weight and total length of first and second-order lateral roots. However, there was no significant difference in the total number of first-order lateral roots among treatments. Total root length was significantly correlated with root dry weight as shown in Figure 2.

Total dry weight accumulated by soybean plants from the +N treatments was nearly double that of the -N plants. The DMD within the plant was also affected by the N regime. Plants dependent upon symbiotically

TABLE 1. Effect of combined nitrogen and rhizobial inoculation on the dry matter distribution and root development in 23 day old soybean.

Treatments		Nodule dry weight	Root dry weight	Top dry weight	Nodule number	Primary lateral roots	Total root length	Root + nodule: shoot ratio	Root: total plant ratio
		g/plant			number/plant		cm/plant		
-N	-I	0.004	0.16	0.49	4	85	514	0.34	0.25
	+I	0.062	0.14	0.54	47	92	422	0.37	0.19
+N	-I	0.002	0.22	1.14	5	83	807	0.19	0.16
	+I	0.015	0.19	1.06	61	80	646	0.20	0.16
Source									
Inoc.		***	n.s.	n.s.	***	n.s.	**	n.s.	n.s.
Nitrogen		***	**	***	*	n.s.	***	***	***
N x Inoc.		**	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.

*,**, *** indicate significance at 0.05, 0.01, and 0.001 levels, respectively.

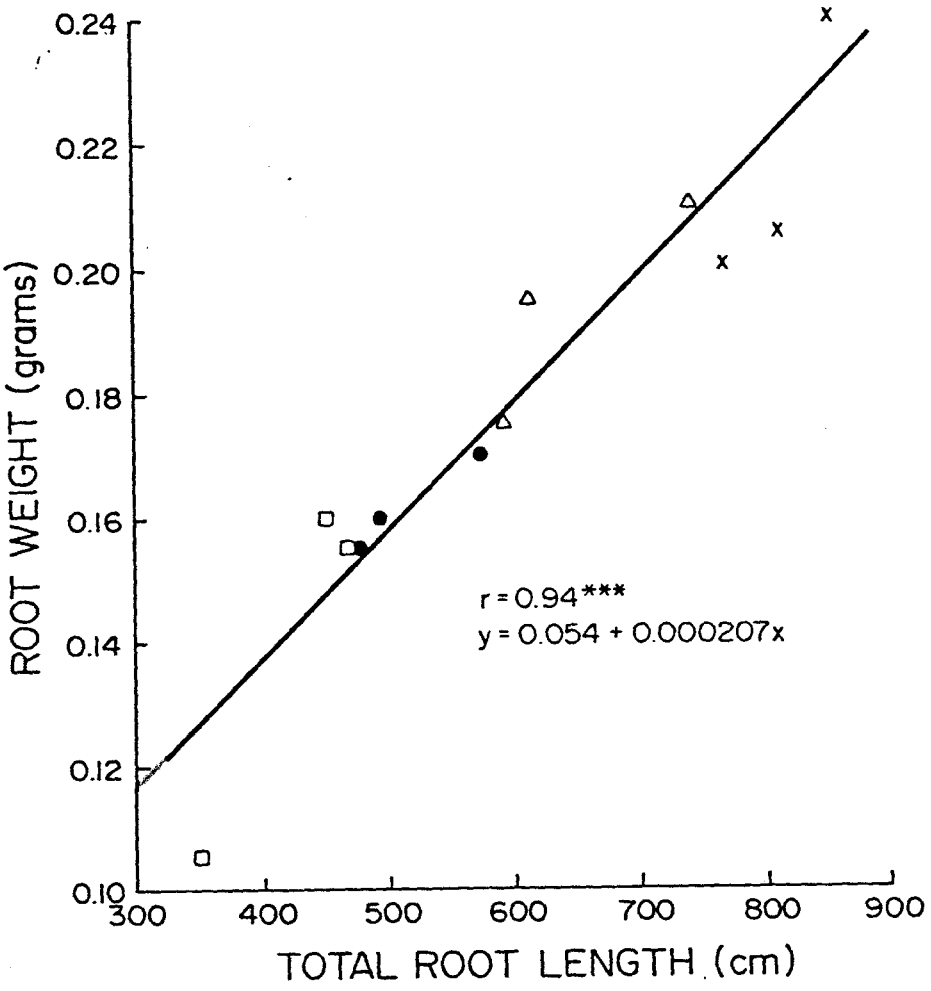


Figure 2. Relationship between root dry weight and total root length in 21 day old soybean as affected by rhizobial inoculation and combined nitrogen. The four treatments include: -N+I (▫), -N-I (●), +N+I (△), and +N-I (x).

produced N accumulated relatively more dry matter in their underground parts as shown by the root+nodule:shoot dry weight ratio data in Table 1. The nodules on these roots comprised 28% by weight of the below ground portion of the plant. If the root dry weight is compared with the total plant dry weight as in the root:total plant dry weight ratio, it is evident that there is a relatively larger root mass in the -N-I plants. There were no differences in the root+nodule:shoot dry weight ratio or the root:total plant dry weight ratio between plants in the +N-I and +N+I treatments.

Experiment 2.

The N regimes for +N plants were initiated 16 days after planting. Day 16 was chosen because it coincided with the appearance of green leaf-veins in the first trifolioate leaf indicating that effective transport of symbiotically fixed N to the shoots had begun. Plants grown in the P_5+N and P_6+N nutrient solutions developed flushing branches from the axils of cotyledon, primary and subsequent trifolioate leaves. The -N plants grown at the same P levels developed few axillary branches.

Brown necrotic spots began to appear on the expanding second trifolioate leaf (day 19) of plants grown in the P_1, P_2, and P_3 nutrient solutions with and without N. This necrotic spotting was most severe on the youngest leaves. However, these deficiency symptoms did not appear on the third trifolioate leaf and thereafter the expression of P deficiency symptoms occurred only on the older leaves as marginal and interveinal chlorosis which had developed into marginal browning at harvest. Growth of plants at the 3 lowest P levels was severely stunted

regardless of the N source. Phosphorus deficiency symptoms were only slightly apparent at harvest on some plants in the P_4 treatments. First flowering occurred 26 days after planting and there was no effect of P or N on the time of flowering. Small pods were present on all plants at harvest.

The dry matter yield was significantly affected by both P supply and N source (Fig. 3). The highest dry matter yield was produced by plants grown with 2.0 ppm P in the nutrient solution in both the +N and -N regimes. The NxP interaction was highly significant. The presence or absence of N in the nutrient solution did not significantly affect the dry matter yield at the 4 lowest P levels; however, the dry matter yield of plants in the P_5-N and P_6-N treatments was only 68% and 57%, respectively, of the comparable +N phosphorus treatments. The presence of 5.0 mM N in the nutrient solution was sufficient to inhibit nitrogenase activity, as measured by acetylene reduction, at all but the highest P level (Fig. 4). Measurement of the NO_3-N depletion from the nutrient solution during the 24 hour period following the final nutrient solution change indicated that the plants in the P_6+N treatment were the only plants that were active in the uptake of NO_3-N from the nutrient solution. The NO_3-N concentration in the nutrient solutions of plants growing at the 5 lowest P levels did not change significantly. This indicates that the N supply in the pot system was not sufficient to completely inhibit nitrogenase activity in 5 week old soybean grown in the highest P treatment. Nitrogenase activity in the -N phosphorus treatments was significantly correlated with the total dry matter yield ($r=0.97$) and reached a maximum rate of 87 μM C_2H_2

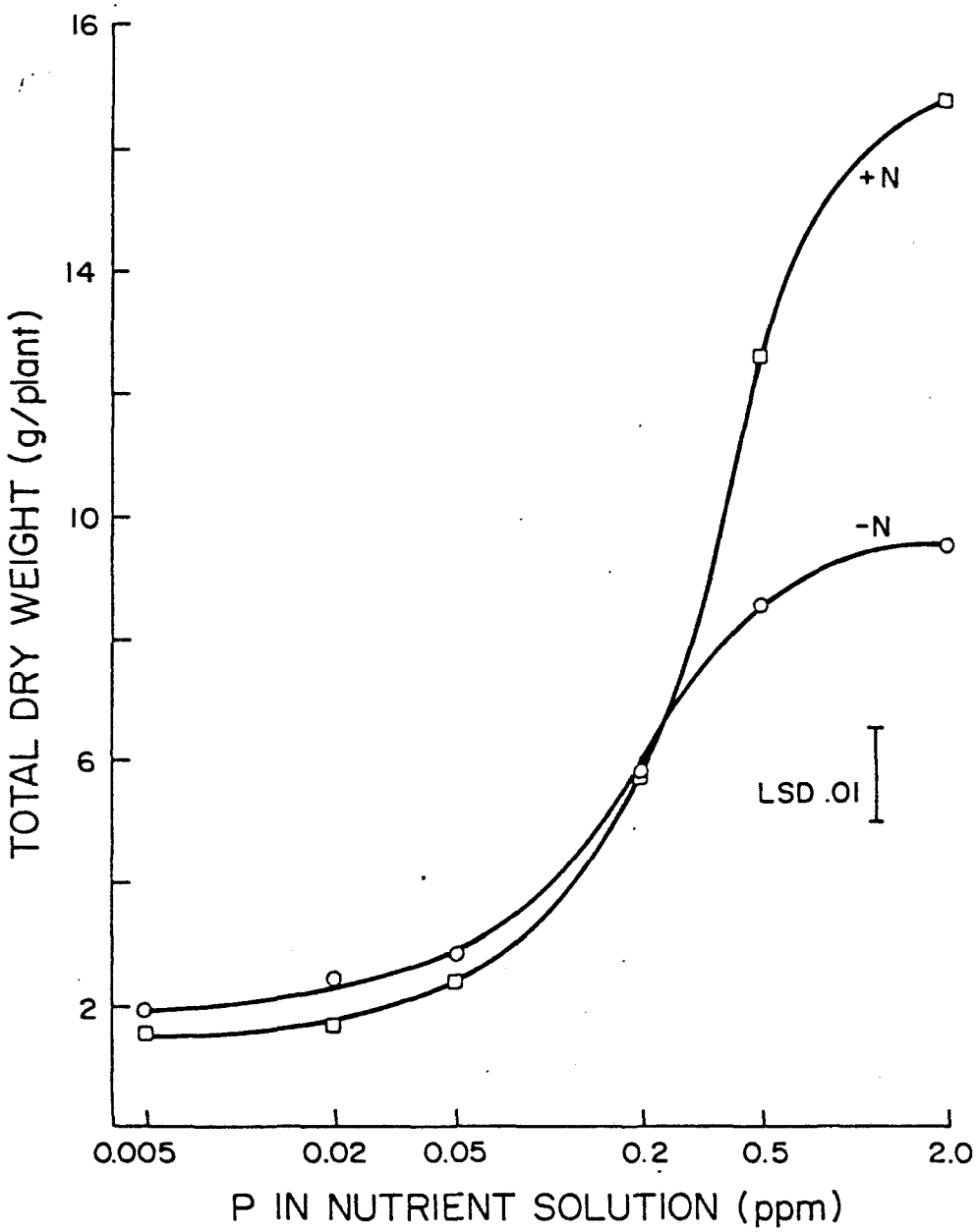


Figure 3. Growth of soybean as affected by the phosphorus concentrati in nutrient solutions with and without combined nitrogen.

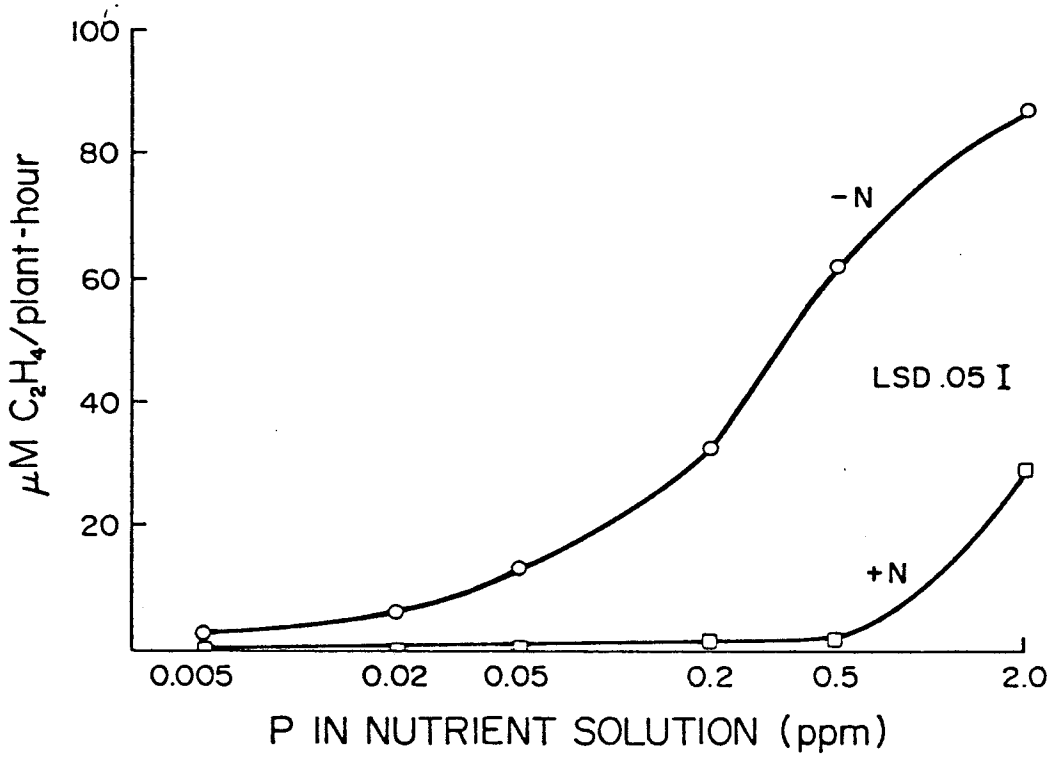


Figure 4. Acetylene reduction rate of 35 day old soybean plants as affected by the phosphorus concentration in nutrient solutions with and without combined nitrogen.

reduced/plant-hour for plants grown at the P_6 level.

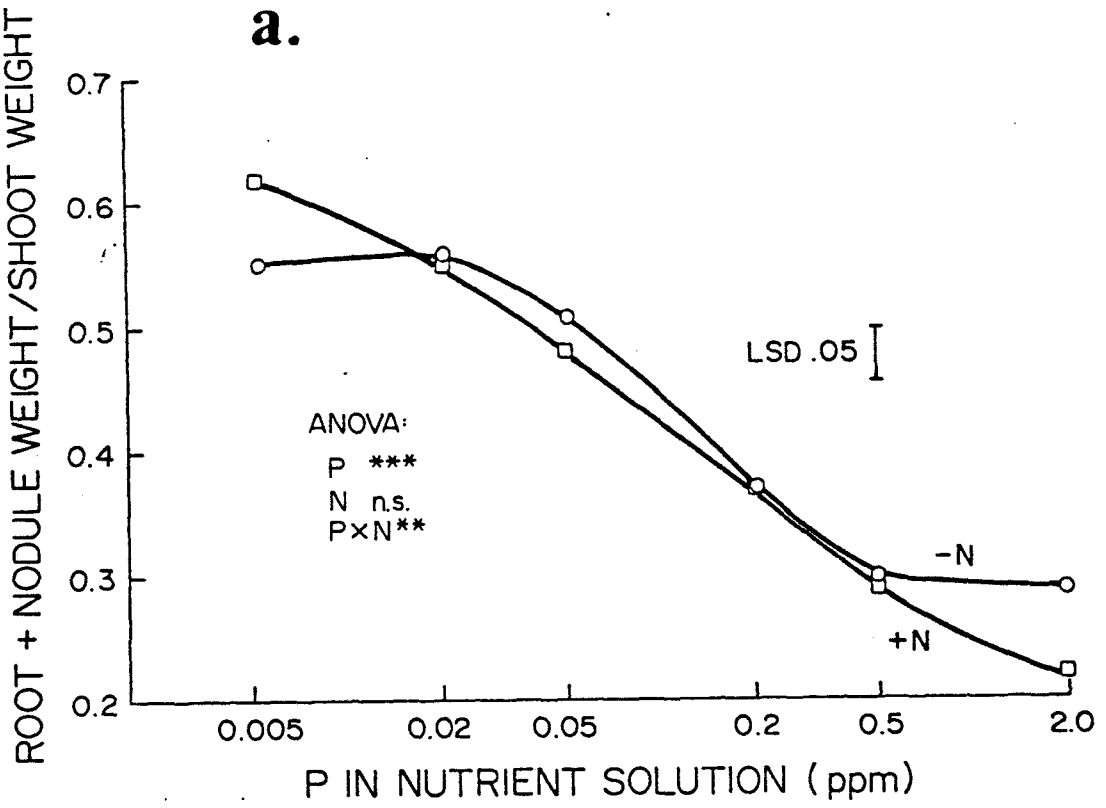
The P supply and the mode of N nutrition affected the DMD within the soybean plant. The data presented in Table 2 indicates three trends. First, the top:total plant dry weight ratio increased as the P supply increased. The mode of N nutrition did not significantly affect this ratio. Second, the root:total plant dry weight ratio in both the -N and +N treatments decreased as the P supply increased although at any given P level there was a larger root:total plant dry weight ratio in plants from the +N treatments. Third, as the P supply increased, the nodule dry weight comprised a larger proportion of the total underground portion of the -N plants. In these -N treatments it is the nodules that show the greatest relative dry weight increase across P treatments. In the +N treatments the top dry weight shows the greatest relative increase as the P supply is increased.

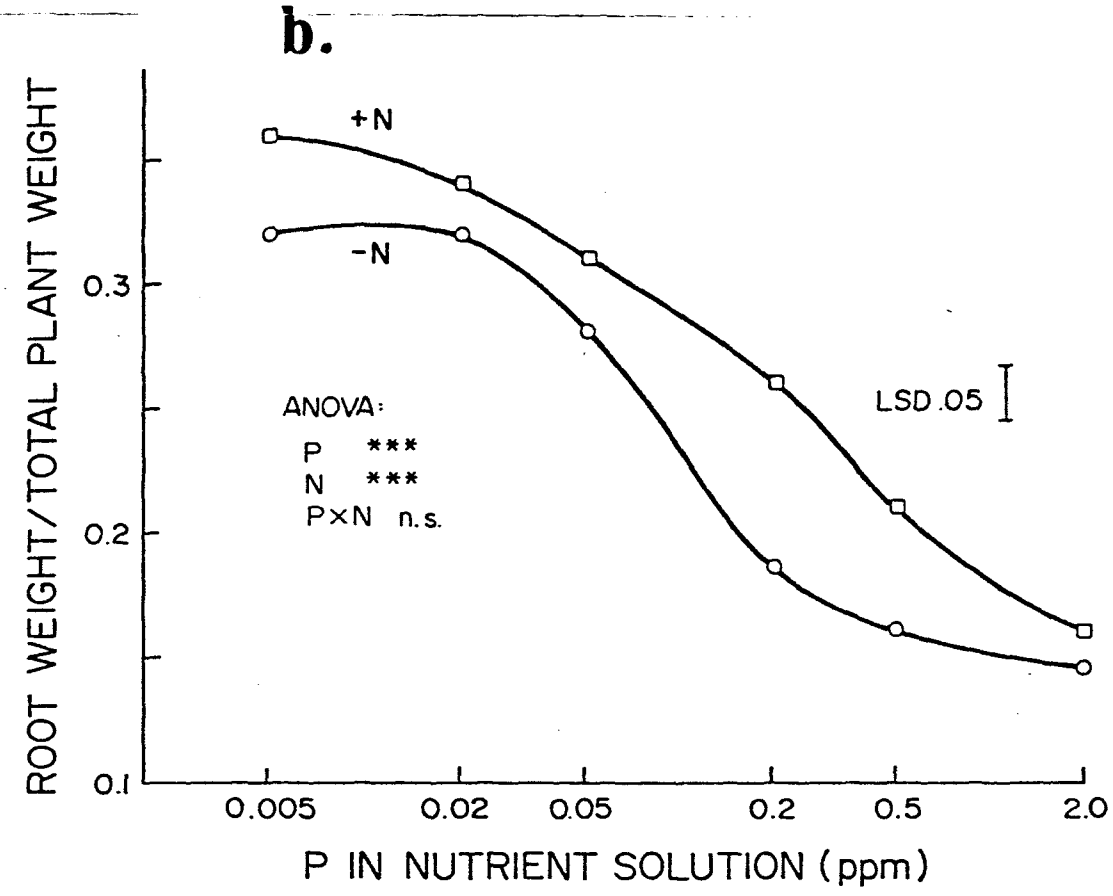
The mode of N nutrition did not significantly affect the relative distribution of dry matter between the shoot and the underground portion of the plant in the intermediate P treatments (P_2, P_3, P_4, and P_5). There was no difference in the root+nodule: shoot dry weight ratio between -N and +N treatments at each of these P levels and this was true regardless of total plant weight (Fig. 5a). However, at any given P level the mode of N nutrition did affect the root:total plant dry weight ratio (Fig. 5b). The effective nodulation of soybean plant roots significantly decreased the root:total plant dry weight ratio of -N plants relative to those in the +N treatments at equivalent P levels and this difference was greatest when the P supply was suboptimal.

Table 2. Dry matter distribution in 35 day old soybean as affected by phosphorous supply and mode of nitrogen nutrition.

P level	N level	Nodule dry weight	Root dry weight	Top dry weight	Nodule: total plant ratio	Root: total plant ratio	Top total plant ratio
ppm P	mM N	g/plant					
0.005	0	0.07	0.60	1.21	0.04	0.32	0.64
	5.0	0.04	0.55	0.95	0.02	0.36	0.62
0.02	0	0.10	0.76	1.55	0.04	0.32	0.64
	5.0	0.04	0.56	1.07	0.02	0.34	0.64
0.05	0	0.16	0.79	1.86	0.06	0.28	0.66
	5.0	0.05	0.72	1.60	0.02	0.30	0.68
0.20	0	0.35	1.23	4.22	0.06	0.21	0.73
	5.0	0.07	1.46	4.21	0.01	0.26	0.73
0.50	0	0.64	1.35	6.57	0.08	0.16	0.77
	5.0	0.15	2.68	9.77	0.01	0.21	0.78
2.00	0	0.81	1.33	7.36	0.09	0.14	0.78
	5.0	0.43	2.65	13.82	0.02	0.16	0.81
Bayes $LSD_{.05}$		0.05	0.18	0.86	0.01	0.02	0.02

Figure 5. The (a) root+nodule:shoot and (b) root:total plant dry weight ratios of 35 day old soybean plants as affected the concentration of P in the nutrient solution with and without combined N.


a.
ROOT + NODULE WEIGHT/SHOOT WEIGHT
0.7
0.6
0.5
0.4
0.3
0.2
LSD .05
ANOVA:
P ***
N n.s.
P×N**
-N
+N
0.005
0.02
0.05
0.2
0.5
2.0
P IN NUTRIENT SOLUTION (ppm)


b.
ROOT WEIGHT/TOTAL PLANT WEIGHT
0.3
0.2
0.1
+N
-N
ANOVA:
P ***
N ***
P×N n.s.
LSD .05
0.005
0.02
0.05
0.2
0.5
2.0
P IN NUTRIENT SOLUTION (ppm)

DISCUSSION

The soybean plant can meet its N requirements for growth in two ways: (1) it may absorb N that is available in the soil, and (2) atmospheric N_2 may be reduced by the Rhizobia in the nodules and assimilated by the plant. For both experiments, two N treatments were employed to establish these two distinct modes of N nutrition. In Experiment 1, the +N nutrient solution did not inhibit nodulation in +I treatment although nodules were extremely small, had no visible leghaemoglobin, and did not have significant nitrogenase activity. In Experiment 2, both -N and +N plants were inoculated at planting and the +N regime was not initiated until 16 days after planting. The +N plants were inoculated to provide an internal control since it can be assumed that if nodule mass and nitrogenase activity were severely inhibited, the N supplied in the nutrient solution was sufficient to meet the requirement of the soybean plant during growth (Allos and Bartholomew, 1959). The data presented in Table 2 and Figure 4 show that there was a relatively small nodule mass and nitrogenase activity in the +N plants at all but the highest P level. It is concluded that the +N and -N treatments in both experiments provided suitable growth conditions to assess the effect of these two modes of N nutrition upon DMD and root development in the soybean plant with the exception of the P_6+N treatment.

In Experiment 1 the evidence presented suggests that there is an inverse relationship between nodule mass and total root length. This could not be attributed to a reduction in the number of first-order

lateral roots. Rather, the observed decrease in total root length was caused by a reduction in lateral root extension. These findings suggest that the "focus hypothesis" i.e. that nodules are formed at lateral root initiation sites, as proposed by Nutman (1948) for red clover, is not applicable to soybean.

In the inoculated, -N treatments at optimum P levels in both Experiments 1 and 2, nodule dry weight comprised 9% of the total plant dry weight. In the P_1-N treatment of Experiment 2, nodule dry weight was equivalent to 12% of the root dry weight. This increased to 61% in the P_6-N treatment. It is apparent from the data in Table 2 that for soybean plants dependent upon symbiotically fixed N as their sole N source, a decrease in the P supply affected nodule dry weight relatively more than either shoots or roots. The nodules of +N plants comprised a much smaller proportion of both total plant dry weight and root dry weight.

Brouwer (1962) hypothesized that if there is a suboptimal nutrient supply, the growth of shoots will be checked sooner than that of roots because the latter are closer to the source of the deficient nutrient. For soybean, P stress does increase the root:total plant dry weight ratio in both the -N and +N treatments (Fig. 5b) as predicted by this hypothesis. Brouwer (1962) also suggested that there is a "functional equilibrium" in the partitioning of photosynthate between the shoot and root and that this equilibrium is altered by a suboptimal nutrient supply. It is clear that there was an equivalent partitioning of dry matter between the shoot and the underground portion of the plant regardless of N source at the P_2, P_3, P_4, and P_5 phosphorus levels

(Fig. 5a). At lowest and highest P levels, the partitioning of dry matter between the shoot and the underground portion of the plant differed depending upon the mode of N nutrition. For soybean plants dependent upon symbiotically fixed N, photosynthate translocated to the below-ground portion of the plant was utilized for both the growth and function of the root and the growth and function of nodules. Consequently the root:total plant dry weight ratios in the -N treatments were always less than in the +N treatments at equivalent P levels (Fig. 5b). This indicates that the nodules and the roots were competitive sinks for the photosynthate that was translocated from the shoot to the underground portion of the plant during the establishment and vegetative stages of growth. Thus for the N-fixing soybean plant, we conclude that a second functional equilibrium was operative, namely, the partitioning of photosynthate between the root and nodules and this equilibrium is more sensitive to P stress than the partitioning of photosynthate between the shoot and the underground portion of the soybean plant (Table 2). Lawn and Brun (1974) demonstrated a similar competitive sink relationship between the nodules and developing pods during pod-fill in soybeans. Ryle et al (1978) in their work with soybean found that root growth ceased and N fixation declined sharply at the onset of rapid seed development, 55-60 days after germination. They suggested that the flow of photosynthate to both roots and nodules is controlled by the same physiological mechanism. The results from the experiments reported here support this hypothesis.

An inverse relationship between soybean root development and nodule mass should have important agronomic implications. Other growth

characteristics being equal, an extensive root system enables the plant to exploit the soil reserves of moisture and nutrients efficiently. The difference in the root:total plant dry weight ratio between N-supplied and N-fixing soybean plants was greatest when the P supply was suboptimal. Therefore, it is possible that effectively nodulated soybeans grown on an N-deficient soil would be more susceptible to growth limitations caused by deficiencies of essential mineral nutrients or drought compared with their N-fertilized counterpart, especially during early growth. Fertilizer use efficiency for macronutrients other than N might also be affected by the mode of N nutrition. In a field experiment, Ham and Caldwell (1978) reported that nonnodulating soybeans fertilized with 100 kg N/ha and 35 kg P/ha had nearly double the phosphorus fertilizer use efficiency of nodulated soybeans fertilized at the same P rate but with no N fertilizer applied. It is possible that the more efficient utilization of P fertilizer by the nonnodulated soybean fertilized with N was caused by the development of a more extensive root system during early crop growth, before the applied P fertilizer had completely reacted with the soil. The data from Experiment 1 in this study showed that there was indeed a significant early growth response to supplied N in root dry weight, total root length, and top dry weight of 21 day old soybean (Table 1).

In experiments discussed in this paper we have shown that nodulation, P stress, and the mode of N nutrition affects soybean root development and dry matter distribution. However, we have compared these relationships at the two extreme modes of N nutrition only. Most field-grown soybeans derive N both from the soil and from symbiotic N

fixation. It should be worthwhile to study these relationships at intermediate levels of N supply which more closely approximate N availability in the soil. A better understanding of the effects of nodulation, nutrient stress and N availability on soybean ontogeny could lead to improved husbandry practices in the field.

CHAPTER IV

THE PHOSPHORUS NUTRITION OF TWO GRAIN LEGUMES AS AFFECTED BY THE MODE OF NITROGEN NUTRITION.

I. ESTABLISHING N-DEFICIENT AND N-LUXURIANT SOIL CONDITIONS

INTRODUCTION

Soybean and cowpea may derive N from soil sources and from the symbiotic fixation of atmospheric N_2. The relative contribution of soil-derived N or symbiotically-derived N to total plant N varies considerably and is primarily dependent upon the availability of soil N. For soybeans grown on N-deficient soil, the proportion of symbiotically derived N can comprise 70 to 80% of the total plant N when highly effective strains of Rhizobium japonicum are present (Weber, 1966; Bezdicek et al, 1978). On soils which have large N-supplying capabilities or which have received large N-fertilizer applications (N-luxuriant soils) the symbiotic contribution to total plant N comprised 1 to 30% of the total (Weber, 1966; Harper, 1974; Hardy, 1976). In pot studies, Summerfield et al (1977) found that N-fixation in cowpea also decreased under N-luxuriant conditions.

Several field experiments with soybean have shown that large additions of carbonaceous material to the soil can cause an increase in the proportion of total plant N derived from symbiotic N fixation. Sears and Lynch (1951) incorporated oat straw at a rate of 11,000 hg/ha and found that symbiotically-derived N comprised 66% of the total plant N in soybeans grown under these modified soil conditions. By contrast, soybean plants grown on unamended soil derived only 20% of their total

N from symbiotic N-fixation. Weber (1966) incorporated ground corn cobs at 45,000 kg/ha and reported the symbiotic input to be 72% and 40% of the total plant N for the modified and unamended soil, respectively. In both field experiments it was assumed that the incorporated material caused a partial immobilization of the available soil N thereby creating N-deficient soil conditions which were more conducive to symbiotic N fixation. However, the availability of soil N under modified soil conditions was not measured directly.

Under most field conditions (adequate soil aeration and pH $\geq$5.4) the rate limiting step in soil N mineralization is ammonification, the conversion of organic-N to ammonium-N. Once ammonification has occurred, the oxidation of ammonium to nitrate normally occurs rapidly enough to prevent the accumulation of ammonium ions in the soil. Thus nitrate is the primary source of soil-derived N for most field-grown plants. Nitrogen mineralization rates vary greatly among soils. However, Sanford and Smith (1972) demonstrated that the N mineralization rate is proportional to the total quantity of mineralizable N present in the soil and that the N mineralization rate constant did not differ significantly among most soils when studied under a defined set of environmental conditions. In a subsequent greenhouse pot experiment, Stanford et al (1973) found that the actual soil N availability (as measured by plant N uptake) was well correlated with the cumulative net N mineralized as estimated by the in vitro N mineralization potential method of Stanford and Smith (1972). These findings suggest that net cumulative N mineralization could be a valuable tool in correlating soil N availability with symtiotic N fixation by legumes grown on different soils.

The purpose of the experiments reported in this study was to establish N-deficient and N-luxuriant soil conditions in the field and to correlate the subsequent cumulative net N mineralization in the soil with legume nodulation and N fixation.

MATERIALS AND METHODS

In Vitro Incubation and Pot Experiment with Bagasse.

An in vitro N mineralization experiment was conducted using the method of Stanford and Smith (1972) with some modifications in order to determine the rate of sugarcane bagasse application necessary to immobilize most of the available soil N and also the rate of N addition necessary to creat N-luxuriant soil conditions in the bagasse-treated soil. A composite soil sample was collected after the corn harvest from each P_{1360} plot. The soil was air dried and passed through a 2 mm sieve. Treatments included four rates of ground bagasse additions (0, 0.2, 0.6, and 1.2% on a dry weight basis) combined factorially with four rates of N addition (0, 100, 300, and 900 ppm urea-N), each treatment combination replicated two times. Ground bagasse at the appropriate rate was mixed with 25 g soil (dry weight basis) and 25 g of 40 mesh silica sand and packed in 60 ml leaching tubes with glass wool wads at the bottom and top of the soil-sand mixture. The soil-sand mixture was then loaded with N by leaching each tube with 150 ml of a plant nutrient solution containing urea-N at 0, 167, 500, or 1500 ppm followed by removal of excess solution under controlled suction to 30% water content. The tubes were incubated at 30°C for 3 weeks and leached weekly with

100 ml of the -N plant nutrient solution. After the third leaching, the tubes from the 0.6% bagasse + 300 ppm N treatment were reloaded with another 100 ppm urea-N and a final leaching done after incubation for one more week. The recovery of NO_3-N in the weekly leachates was determined by the cadmium reduction method (APHA, 1975).

A greenhouse pot experiment was conducted to determine if there were detrimental side effects on the growth of either soybean or cowpea caused by incorporation of bagasse in the soil or by the addition of large quantities of urea-N that were required to overcome the bagasse effect. The experiment employed a completely randomized design with five treatments, each replicated twice. Treatments included: the soil with no bagasse or N added and four treatments in which bagasse had been incorporated into the soil at a rate of 0.8% and to which urea-N was applied at four rates (0, 100, 200, and 300 ppm N). Each 12 inch pot contained 5.2 kg limed Haiku soil fertilized with 12.2 g $Ca(H_2PO_4)_2 \cdot H_2O$, 1.7 g K_2SO_4, and 2.5 g $MgSO_4 \cdot 7H_2O$. Water was added to the soil-filled pots to bring the moisture content to 34% (1/3 bar matric suction) and were equilibrated in the greenhouse for 3 days before planting. On one side of the pot three pregerminated seeds of "Clark 63" soybean were planted and inoculated with 10^7 viable rhizobia per seed (TAL strain 379 = USDA 136 b = CB1809). On the other side of the pot three pregerminated seeds of cowpea line TVu 745-P3 were planted and inoculated with 10^7 viable rhizobia per seed (TAL strain 309 = CB756). Pots were weighed daily and watered to restore the soil moisture content to 34%. The plants were harvested 23 days after planting by cutting the stem at the soil surface. Soil was removed from the roots, the roots of cowpea

plants separated from the soybean roots, and placed in separate 2 liter incubation bottles with 5% acetylene. After incubation for 1.5 hours, ethylene production was measured by gas chromatography. Tops were dried at 55°C and weighed.

Field Experiment.

Field investigations were conducted at the University of Hawaii Kuiaha Experiment Site on the island of Maui, Hawaii. The elevation at the site is 320m and mean annual rainfall is 1800 mm. The soil is a Haiku clay (clayey, ferritic, isohyperthermic Humoxic Tropohumult) weathered from basic igneous rock and volcanic ash but presently comprised of well aggregated oxides of iron. Surface soil and subsoil pH was 4.8 before liming. Other soil characteristics are: bulk density, 1.1 g/cc; organic carbon, 3.1%; total N, 0.26%; extractable P (Bray P1), 0.5 ppm; and extractable bases per 100 g soil (extracted with 1.0 M NH_4Cl) include 12.3 meq Ca, 1.4 meq Mg, 2.6 meq K, and 0.4 meq Na.

The field was sprayed with glyphosate herbicide, mowed, dead plant material removed, and tilled with a rotary tiller. One month before planting, the soil was limed with 2100 kg/ha finely ground agricultural lime and 1600 kg/ha dolomitic lime which raised the pH of the surface soil to 5.9. Blanket fertilizer applications of 200 kg/ha K as K_2SO_4, 15 kg/ha Zn as $ZnSO_4$, and 2 kg/ha Mo as MoO_3 were also applied. Six phosphorous treatments were established to provide 0.0015, 0.011, 0.024, 0.070, 0.170, and 0.500 ppm P in the soil solution as estimated by the P sorption isotherm method of Fox and Kamprath (1970); requiring P applications of 0, 400, 620, 960, 1360, and 1880 kg P/ha. These six

treatments are designated P_0 through P_{1880} in the text. Phosphorus was surface broadcast as treble superphosphate, rotary-tilled to 18 cm, and irrigated four times to insure equilibration prior to planting. Plots were 5 m x 10 m and were arranged in a completely randomized block design with three replications.

A uniformity trial was conducted to assess the homogeneity of soil N availability within the field. Corn (Zea mays L.), cv. "H-610", was planted on September 13, 1977. Extractable NO_3-N was determined for each plot from soil samples collected five weeks after planting using 20 g air dried soil extracted with 100 ml 1.0 M KCl leached in 20 ml increments. The NH_4-N was measured colorimetrically using the method described by Mitchell (1972). The NO_3-N in soil extracts was determined as described previously. Phosphate concentration in the soil solution was also measured in the same soil samples after equilibration for six days in 0.01M $CaCl_2$ (Fox and Kamprath, 1970).

The ear leaf was sampled 65 days after planting, dried at 55°C, ground, and 0.5 g digested by a modified Kjeldahl procedure. Total N was determined colorimetrically (Mitchell, 1972) and P content measured from from the same digest using the ascorbic acid phosphomolybdenum-blue method developed by Murphy and Riley (1962) and modified for Kjeldahl digests by Throneberry (1974). Final corn harvest was at 98 days after planting (hard dough stage). Plants were removed from five randomly selected one meter strips within each plot, weighed in the field, and subsampled for moisture content determination (dried at 55°C).

A cowpea and soybean N x P field experiment was conducted using a split-plot randomized block design. The same P plots used in the corn uniformity trial the previous year comprised the mainplots and these

were split into two N-level subplots of 5 m x 5 m each. Bagasse was incorporated into the entire field during the last week in May, 1978 at a rate of 16,000 kg/ha which was equivalent to 0.8% of the surface soil (18 cm) on a dry weight basis. Urea-N was applied to one subplot in each P main plot at a rate of 350 ppm (700 kg N/ha) on May 31, 1978. Blanket fertilizer was applied at the per hectare rate of 100 kg K as KCl, 50 kg Mg as $MgSO_4$, 20 kg B as $NaHB_4O_3$, and 5 kg Zn as $ZnSO_4$ and the field was rotary-tilled. Three 5 meter rows of both "Clark 63" soybean and cowpea line TVu 745-P3 were planted in each subplot on June 6, 1978. Soybean rows were spaced 1 m apart and intrarow spacing was 3.0 cm (333,000 plants/ha). Cowpea rows were spaced 1 m apart and intrarow spacing was 7.5 cm (133,000 plants/ha). Prior to planting, seeds were coated with peat cultures containing highly effective strains of Rhizobium with gum arabic used as a sticking agent. Soybean seeds were coated with a mixed inoculum containing equivalent numbers of Rhizobium strains TAL 378 and TAL 379. Cowpea seeds were coated with a mixed inoculum containing equivalent numbers of Rhizobium strains TAL 169 and TAL 309. Viable plate counts made from coated seeds indicated that there were 10^7 viable rhizobia on both soybean and cowpea seeds at planting. These inoculation rates are approximately 40 times higher than those required for maximum soybean nodulation in soil (Burton and Curley, 1965; Weaver and Frederick, 1972). Supplemental applications of 100 and 50 ppm urea-N (equivalent to 200 kg and 100 kg N/ha) were side-dressed at four and six weeks after emergence, respectively. Overhead irrigation was used to supplement rainfall whenever necessary to maintain adequate soil moisture levels during crop growth. Four soybean plants and three cowpea plants were

randomly sampled from the middle border row of each subplot at 32 days (vegetative stage) and 46 days (early pod-fill stage of soybean) after emergence to assess nodulation and acetylene reduction activity under N-deficient and N-luxuriant soil conditions. Plants were dug from the soil, the stem cut at the soil surface, soil removed from the root system and placed in 2 liter incubation bottles. Acetylene reduction activity was determined as described earlier. The nodules were removed from roots. Shoot, roots, and nodules were dried at 55°C and weighed. Plants were also sampled 60 days after emergence to visually assess nodulation.

Nitrogen availability in the N-deficient and N-luxuriant subplots was monitored during crop growth. Soil samples were collected from the -N subplots at emergence and again four weeks after emergence. The +N subplots were sampled once at 3 weeks after emergence. Soil samples were air dried and screened as described earlier. Composite samples were made by combining all replicate soil samples from subplots of the two lowest P levels (P_0 and P_{400} treatments), the two intermediate P levels (P_{620} and P_{960} treatments), and the two highest P levels (P_{1360} and P_{1880} treatments). The composite soil samples were mixed with silica sand and packed in leaching tubes as before, each replicated three times. Water was added to each tube to bring the water content of the soil-sand mixture to 30%. The tubes were incubated at 30°C immediately with no initial leaching or loading with N. The -N subplot composite samples collected at emergence were leached at 2, 3 and 4 weeks after incubation began. The -N subplot composite samples collected four weeks after emergence were leached after 1, 2, 3, 4, 7, and 8 weeks.

Total cumulative net N mineralization in the -N subplots was estimated by adding the NO_3-N recovered during the 4 week incubation of soil samples collected at emergence plus the NO_3-N recovered during the 8 week incubation of soil samples collected 4 weeks after emergence. The +N subplot composite samples collected 3 weeks after emergence were leached at 1, 2, 3, 4, 5, 8 and 9 weeks and cumulative net N mineralized summed for the 9 week incubation period. The subsoil (18 to 36 cm depth) was also sampled in the -N subplots and a composite sample made from each block. Leaching tubes were prepared, incubated, and leached at 1, 2, 3, and 4 weeks. Leachates from all composite soil samples were analyzed for NO_3-N and NH_4-N as described previously. A sample of sugarcane bagasse was analyzed for N and P content as described earlier.

Soybean plants from the N-deficient subplots matured 3 days earlier than those in the N-luxuriant subplots. Consequently the soybeans in the N-deficient subplots were harvested 81 days after emergence and the N luxuriant soybeans 3 days later. At harvest soybean plants from 2 meters of the middle row in each subplot were cut at the soil surface and the number of plants counted. The plants were dried at 55°C, seed and haulm yields determined, and subsamples ground for N and P analysis as previously described.

Cowpea was harvested from both the N-deficient and N-luxuriant subplots 90 days after emergence. Plants from 2 meters of the middle row in each subplot were cut at the soil surface, counted, fresh weight recorded, the plant material chopped, and two subsamples taken; one to determine moisture content and another for N and P analysis. At harvest there were mature dry pods, green pods, and flowers present on plants in all treatments.

RESULTS

In Vitro Incubation and Pot Experiment with Bagasse.

Results from the in vitro N mineralization experiment indicated that bagasse incorporation immobilized most of the available soil N during the three week incubation period. The cumulative net N mineralized in bagasse-treated soil which did not receive urea-N was less than 2 ppm NO_3-N (Fig. 6). This was equivalent to 4% of the NO_3-N recovered from the unamended soil. The data also show that available soil N is immobilized over a wide range of bagasse application rates. As expected, addition of urea-N to the soil increased the amount of N mineralized during the three week incubation and increased the amount of bagasse required to inhibit net N mineralization (Fig. 6). When, after the third week, 100 ppm urea-N was reapplied to soil which initially had been treated with 0.6% bagasse and 300 ppm N, all of the applied N was recovered as NO_3-N in the leachate collected after one more week of incubation. At the end of the experiment there was no significant change in soil pH in any of the treatments.

In the greenhouse pot experiment both soybean and cowpea plants grown in the -N, bagasse-treated soil became increasingly chlorotic between 10 and 15 days after planting. By day 16 the leaf-veins on these plants began to turn green and at harvest the leaf color had regained normal color. There was no chlorosis on plants in any of the four other treatments although the plants in the bagasse-treated soil which received 300 ppm N appeared darker green than plants in the other treatments. In bagasse-treated soil, both soybean and cowpea top

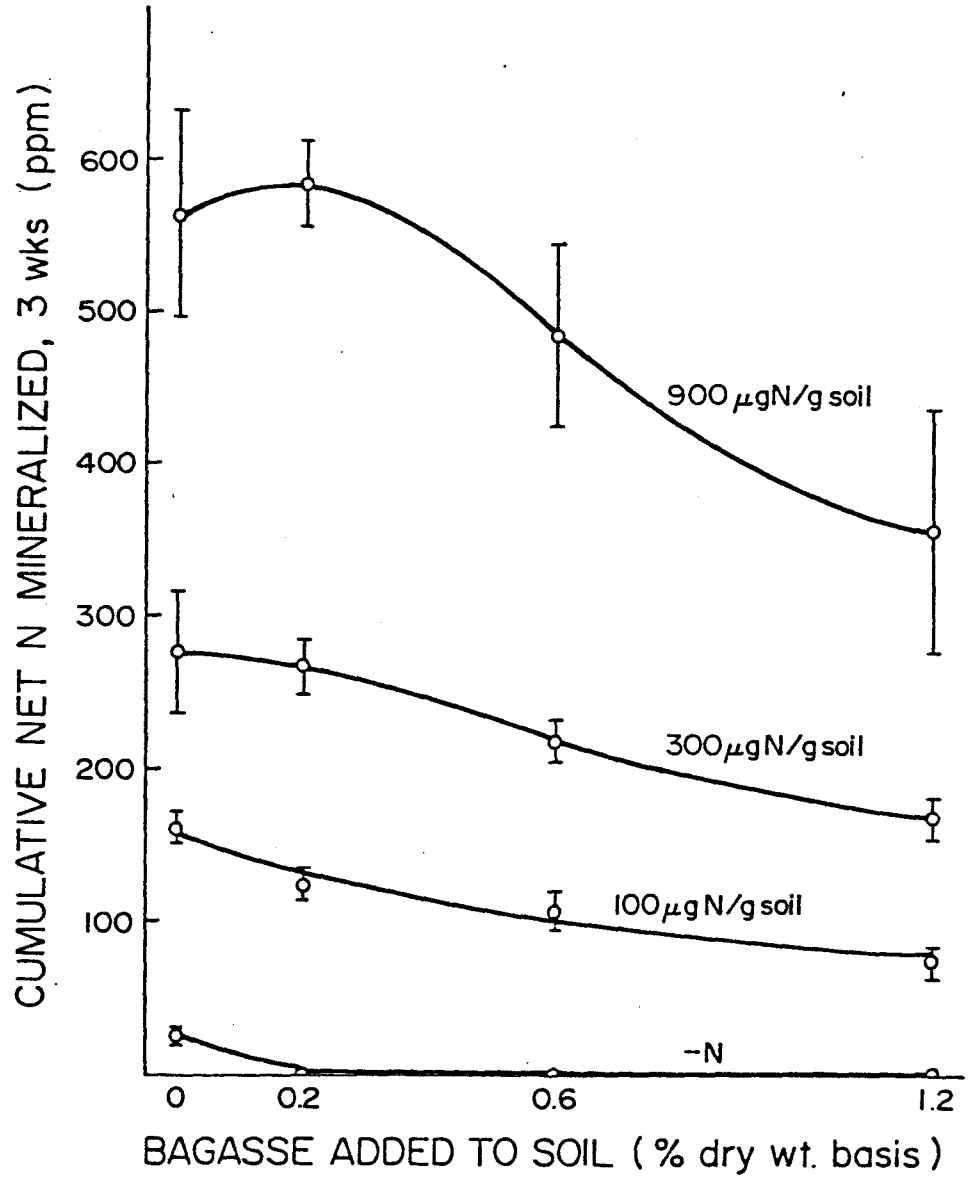


Figure 6. Cumulative net N mineralization in the Haiku surface soil as affected by bagasse incorporation and urea-N additions. The bar intervals represent $\pm 2s_{\bar{x}}$.

weights increased as the level of applied urea-N increased. There was a five-fold increase in the top weight of cowpea at the highest N level over the -N treatment. The comparable increase for soybean was slightly more than two-fold (Fig. 7). There did not appear to be any deleterious effects of bagasse or urea-N additions upon the germination or growth of either cowpea or soybean when bagasse or urea-N was incorporated before planting. The top weight of both cowpea and soybean grown in unamended soil was greater than that in the -N, bagasse-treated soil but less than that in the bagasse-treated soil to which 100 ppm N had been applied.

The acetylene reduction activity was highest for both cowpea and soybean grown in bagasse-treated soil that received 100 ppm (Fig. 8), followed by the unamended soil, the -N, bagasse-treated soil, and was lowest in soils to which high levels of urea-N had been applied. In the bagasse-treated soil to which 300 ppm N had been applied, the acetylene reduction rates were less than 10% of the rate in the -N, bagasse-treated soil even though the N-luxuriant plants were much larger.

Field Experiment.

The corn uniformity trial demonstrated that the soil N availability in the field was extremely heterogeneous. Growth of corn varied considerably within P plots and there was a definite gradient across the field. For instance, the yield of corn from the P_{1880} treatment in block III was lower than that from the P_0 treatment in block I. There was a highly significant block effect ($P<0.001$) and a significant response to applied P fertilizer ($P<0.01$). Regression analysis showed

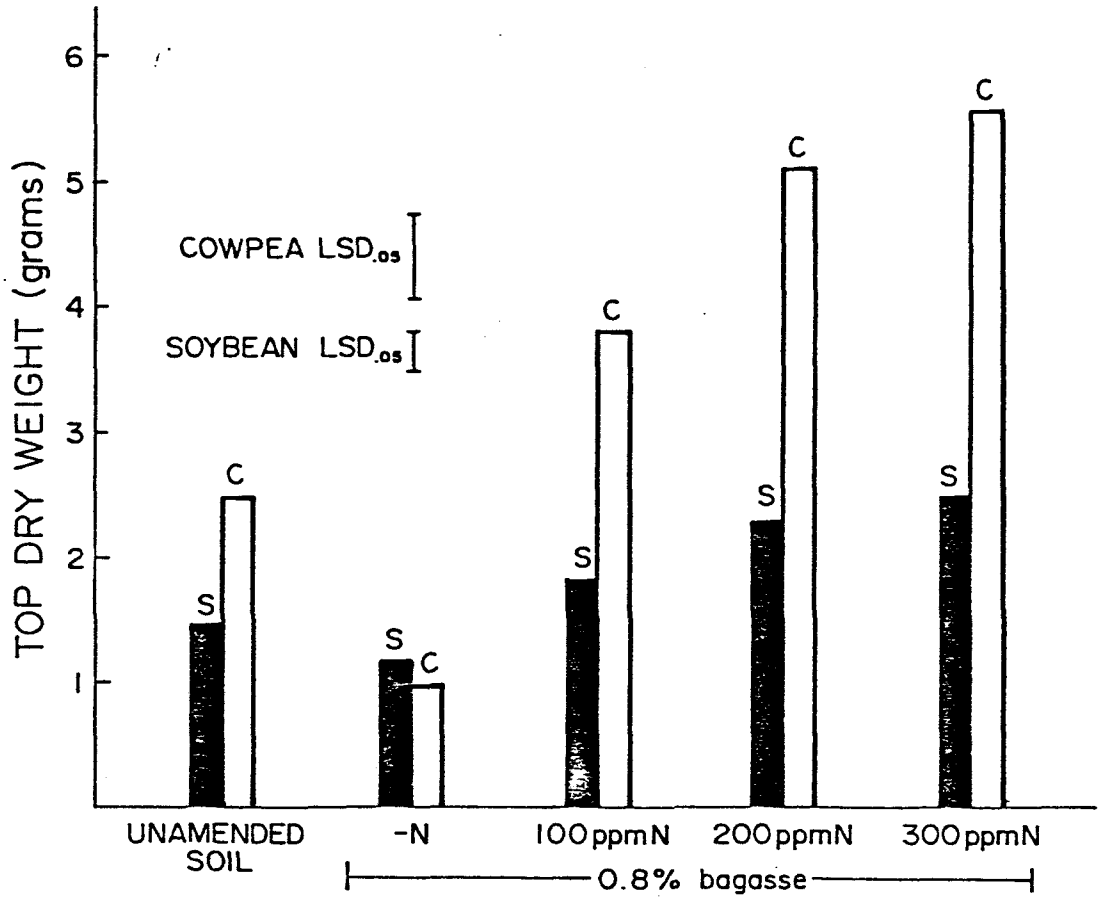


Figure 7. Top growth of 23 day old soybean and cowpea as affected by bagasse incorporation and rate of urea-N application to the Haiku surface soil.

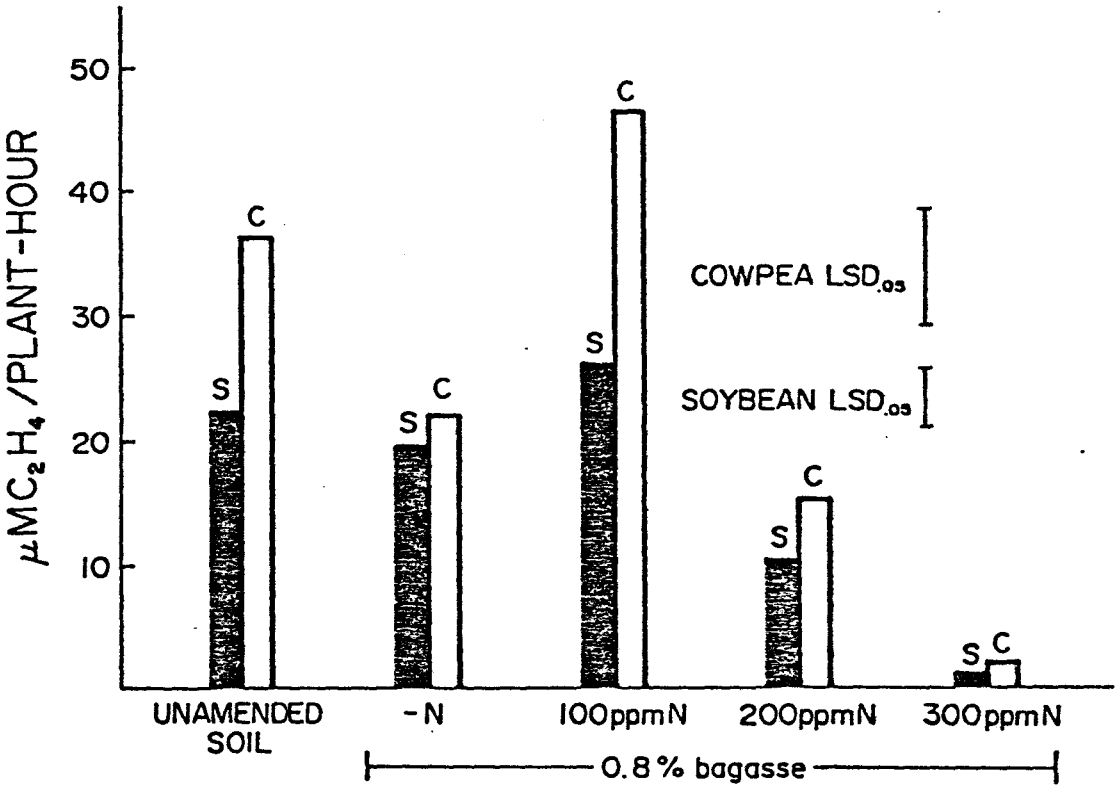


Figure 8. Acetylene reduction rate of 23 day old soybean and cowpea plants as affected by bagasse incorporation and rate of urea-N addition to the Haiku surface soil.

that the P and N content of the ear leaf were not correlated with yield whereas there was a highly significant correlation between extractable soil NO_3-N and yield of corn ($P < 0.01$).

The early growth of both soybean and cowpea in the N-deficient and N-luxuriant subplots in the field was similar to the growth in the pot experiment at comparable N levels. There was a definite inhibition of nodule development on both soybean and cowpea sampled from the N-luxuriant subplots at all P levels. Nodule weight of soybean plants sampled from the N-luxuriant subplots at 32 and 46 days after emergence was less than 11% of the nodule weight of plants from the N-deficient subplots at equivalent P levels (Table 3). Similarly, the acetylene reduction rate of the N-luxuriant soybean plants was less than 7% of the rate occurring in plants grown under N-deficient conditions at equivalent P levels (Table 3). The roots of cowpea plants grown on the N-luxuriant subplots were free of nodules at both sampling times and acetylene reduction activity of these plants was insignificant. Cowpea plants from the N-deficient subplots were well nodulated and both nodule weight and acetylene reduction rate were significantly higher on plants from the P-fertilized treatments. At 60 days after emergence, the inhibition of nodule development on N-luxuriant soybean and cowpea plants was still visibly evident.

The *in vitro* incubations of composite soil samples from the different P treatments indicated that there were no differences among the net N mineralization rates at different P levels from the same N treatments. Consequently, all data presented are the average for all three composite samples from N-deficient or N-luxuriant subplots. The

Table 3. Nodulation and acetylene reduction activity of soybean grown under N-deficient and N-luxuriant soil conditions at different soil P levels.

P treatment		32 days after emergence		46 days after emergence	
		C_2H_2 reduction	Nodule weight	C_2H_2 reduction	Nodule weight
		MC_2H_4/plant-hr.	g/plant	MC_2H_2/plant-hr.	g/plant
P_0	-N	2.0	0.04	4.9	0.05
	+N	0.1	tr	tr	tr
P_{400}	-N	15.2	0.14	23.8	0.20
	+N	1.0	0.01	1.1	0.01
P_{620}	-N	18.9	0.17	36.8	0.32
	+N	1.0	0.02	1.0	0.01
P_{960}	-N	19.4	0.17	35.4	0.30
	+N	0.6	0.01	1.4	0.01
P_{1360}	-N	24.0	0.19	44.0	0.39
	+N	0.9	0.02	1.7	0.02
P_{1880}	-N	22.6	0.19	56.0	0.38
	+N	0.5	0.01	1.5	0.02
*$LSD_{.05}$	(N)	2.7	0.03	12.1	0.07
**$LSD_{.05}$	(NxP)	3.0	0.03	11.9	0.06

* Used to compare different N treatments at the same P level

** Used to compare different N treatments at different P levels or the same N treatment at different P levels.

rate of net N mineralization in soil from the -N subplots was extremely low (0.5 ppm N/week) during the first six weeks of incubation (Fig. 9). The bagasse effect declined during the seventh week and in the last four weeks the net N mineralization rate was not different from that in the N-luxuriant subplot soil samples. The initial application of 350 ppm urea-N to the +N subplots (32 days prior to soil sampling) resulted in only 25 ppm net N mineralized during the first week of incubation. Thereafter, net N mineralization occurred at a constant rate of 7.1 ppm per week. The cumulative net N mineralized during the 12 week incubation of the -N soil samples was 37 ppm NO_3-N. The cumulative net N mineralized during the 9 week incubation of the +N soil samples was 83 ppm NO_3-N. There was no detectable NH_4-N in the leachates from any of the incubated soil samples. Subsoil net N mineralization rate averaged 0.4 ppm NO_3-N per week during the four week incubation period.

DISCUSSION

When effective Rhizobia are present, N-deficient and N-luxuriant soil conditions can be defined with respect to the mode of N nutrition that was most prevalent during the growth of the legume. Both nodulation and acetylene reduction activity are inhibited by high levels of available N in the growth medium (Weber, 1966; Latimore et al, 1977; Summerfield et al, 1977). The data from experiments conducted by Allos and Bartholomew (1959) with 5 legume species suggest that when the quantity of N available to a legume exceeds that needed for maximal

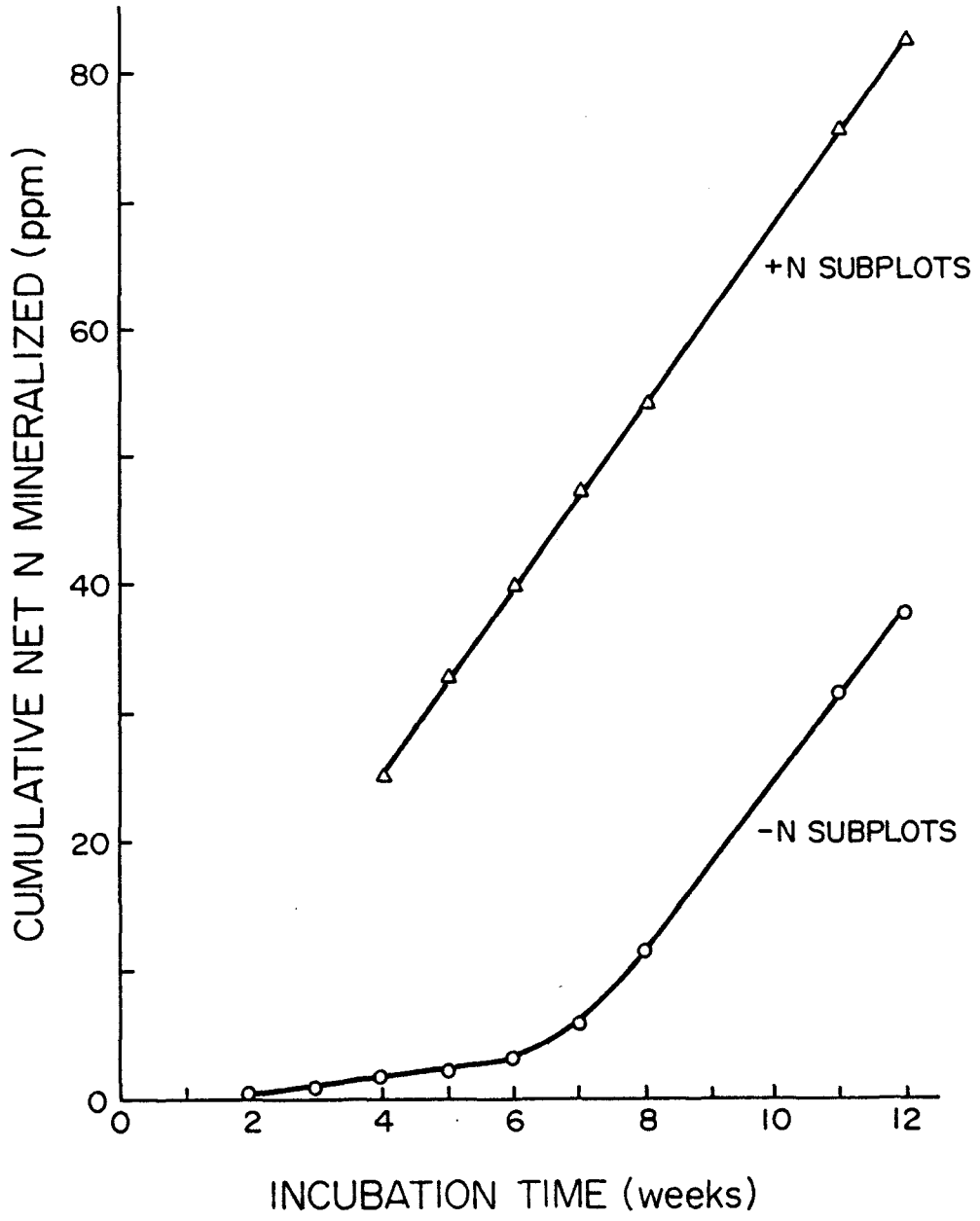


Figure 9. Cumulative net N mineralization in incubated Haiku surface soil sampled from N-deficient (-N) and N-luxuriant (+N) subplots during crop growth.

plant growth, N fixation would be completely inhibited. Therefore, in this discussion, N-luxuriant soils are those in which the legume derives most of its total plant N from soil sources. Under N-luxuriant conditions it is implied that nodulation and acetylene reduction would be severely depressed and that these attributes can be used to indirectly assess the N status of the soil. Nitrogen deficient soil conditions would imply that most of the legume's total assimilated N was derived from symbiotic N fixation. Under N-deficient conditions plant nodulation and acetylene reduction activity would be near maximal.

The complexity of soil N relations is sometimes further complicated by the heterogeneity of soil N availability within an experimental plot. The site used in this experiment is a case in point as demonstrated by the variability in the growth of corn not provided N fertilizer in the uniformity trial. In this experiment the yield of corn was not correlated with the established soil P treatment levels but was significantly correlated with the extractable soil NO_3-N levels.

Incorporation of bagasse into the soil appeared to equalize the soil N availability by immobilizing most of the available soil N. Sugarcane bagasse was well suited for this purpose for three reasons. First, the bagasse had a very low N content (0.2%) which is less than half that reported for cornstalks (Sims and Frederick, 1970), ground corncobs (Weber, 1966), wheat straw (Allison and Klein, 1962), and barley straw (Broadbent and Tyler, 1962) which were used to imoobilize soil N in their experiments. Second, the incorporated bagasse was effective in immobilizing most of the available soil N over a wide range of application rates (Fig. 6). This helped to establish uniform

soil N availability throughout the field even though it was not possible to mix the bagasse with perfect uniformity in the field. Third, the particle size of the bagasse obtained from the sugar mill varied from a fine powder up to filamental particles 1 to 3 cm in length. Sims and Frederick (1970) demonstrated that cornstalk pith of particle size 2 mm or less, when mixed with soil, immobilized more soil N and did so more quickly than did larger-sized fractions. However, in soil treated with the finer material, net N mineralization began to occur after 26 days of incubation whereas immobilization continued up to 56 days in soil treated with the coarser material. This would indicate that the range of particle sizes present in the mill bagasse facilitated both a rapid soil N immobilization and a sustained, longer lasting effect.

That incorporation of bagasse created N-deficient soil conditions was demonstrated in the in vitro nitrification experiments, a pot experiment, and a field experiment. Incorporation of bagasse decreased the cumulative net N mineralized by 96% when compared with the unamended soil during a 3 week incubation (Fig. 6). In the pot experiment, the top weight yield of both cowpea and soybean was significantly lower in the -N, bagasse-treated soil than in the unamended soil (Fig. 7). In the field experiment, nodule mass and acetylene reduction activity of both cowpea and soybean plants from N-deficient subplots compared favorably with plants grown in sand culture with a -N nutrient solution at an equivalent developmental stage (unpublished data). The recovery of NO_3-N in the weekly leachings of incubated soil samples collected from N-deficient subplots during crop growth was monitored for 12 weeks. This was approximately equivalent to the crop growth period from emergence

to harvest in the field experiment. It is evident that bagasse incorporation was effective in immobilizing most of the soil N during the first six weeks and that the net N mineralization rate returned to normal levels during the last five weeks (Fig. 4). Cumulative net N mineralization during the 12 week incubation at 30°C was 37 ppm NO_3-N. The extrapolated 12 week value for the subsoil was approximately 4.8 ppm NO_3-N. Standord et al (1973) found that the Q_{10} for the N mineralization rate in 11 soils of diverse origin was not significantly different, the average value was approximately 2. Thus, correcting for the difference between the incubation temperature and the average soil temperature in the field (at 9 cm depth), the total net N mineralization during the 12 week cropping period in the surface and subsoil in the N-deficient subplots was estimated to be 55 kg N/ha. This estimate would tend to overestimate the plant N derived from the soil for two reasons. First, soil moisture levels in the field fluctuated and would not always be at levels considered to be optimal for N mineralization in soil (Stanford and Epstein, 1974). Second, it is probable that there was not a quantitative recovery of mineralized N by soybeans and cowpea plants as there may have been small losses caused by N uptake by weeds, leaching losses, and denitrification. However, if we assume that all of the net N mineralized during crop growth (as measured by the <u>in vitro</u> method) was assimilated by the plants grown in the N-deficient, P_{1880} treatment, the proportion of total plant N derived from the soil would be 20% and 34% for soybean and cowpea, respectively. This indicates that the N derived from symbiotic N-fixation was equivalent to 80% and 66% of the total plant N

in soybean and cowpea, respectively.

Nitrogen luxuriant soil conditions could be maintained in the bagasse-treated soil by large additions of urea-N before and during crop growth. The in vitro N mineralization experiment indicated that when an initial application of 300 ppm urea-N was mixed in soil treated with 0.6% bagasse, 220 ppm NO_3-N was recovered in the weekly leachings during the 3 week incubation (Fig. 6). However, 94% of this total NO_3-N was recovered in the leachates collected after the first 2 weeks of incubation. In the leachate collected after the third week, only 12 ppm NO_3-N was recovered indicating that the soil was no longer N-luxuriant. When 100 ppm urea-N was reapplied after the third week, there was a quantitative recovery of the applied N as NO_3-N after one further week of incubation. Thus, it appeared that reapplications of urea-N during crop growth were necessary to maintain soil N availability at high levels on the bagasse-treated soil. The greenhouse pot experiment showed that an initial application of 300 ppm urea-N to soil treated with 0.8% bagasse was sufficient to maintain N-luxuriant soil conditions through 23 days of growth as demonstrated by the extremely low acetylene reduction activity on both cowpea and soybean plants from this treatment. In the field experiment, soil was collected from the N-luxuriant subplots 32 days after the initial 700 kg urea-N/ha application. After the first week of incubation, 25 ppm NO_3-N was recovered in the leachate indicating that more than 90% of the initially applied urea-N had been immobilized, denitrified, or leached from the surface soil (Fig. 9). Therefore, to maintain N-luxuriant conditions in the field, 100 and 50 ppm urea-N were reapplied at 4 and 6 weeks

after emergence, respectively. Thus, the total amount of N applied to the N-luxuriant subplots was 1000 kg/ha. The severe inhibition of nodulation and acetylene reduction found in soybean (Table 3) and cowpea plants grown on the +N subplots indicated that these plants were primarily dependent upon soil derived N to meet their total N demand. The cumulative net N mineralization in soil from the N-luxuriant subplots during the 9 week incubation equivalent to the crop growth period from 3 weeks after emergence to harvest was 83 ppm NO_3-N. Corrected for the incubation temperature effect and contribution from the subsoil included, this was equivalent to 115 kg N/ha. The results from the first in vitro experiment indicated that urea-N reapplied to the +N subplots in the field would be rapidly nitrified and that there would be little immobilization by the residual bagasse. Thus, the estimated total available N in the N-luxuriant subplots in the field during the last 9 weeks of crop growth was approximately 415 kg N/ha. This estimate does not include the N mineralized during the first 3 weeks after emergence because plant N uptake during this early stage of growth would be relatively insignificant. The apparent efficiency of N utilization from mineralization and fertilizer sources during this 9 week period was 90% and 57% for soybean and cowpea, respectively. For soybean, the low acetylene reduction activity measured on plants from the N-luxuriant subplots indicated that a small amount of N-fixation was occurring. This would explain the unusually high apparent N utilization efficiency estimated for soybean.

The experiments in this study were initiated to chemically characterize and establish N-deficient and N-luxuriant soil conditions in

a field experiment with two legumes. The results indicate that incorporation of carbonaceous material into the soil is an effective way to equalize soil N availability at relatively N-deficient levels, especially during the 7-8 week period immediately following incorporation. Nitrogen luxuriant soil conditions can be maintained throughout the cropping period by large N-fertilizer applications before and during crop growth. There appears to be a good correlation between the net N mineralization occurring in the soil and the mode of N nutrition of cowpea and soybean plants. The measurement of the net N mineralization is a relatively simple and inexpensive method, and the estimates of symbiotically derived N for soybean grown on N-deficient subplots obtained in this study are similar to values reported by other investigators on soils which were known to be N-deficient (Weber, 1977; Bezdicek et al, 1978). Additional field studies are needed to compare estimates of the contribution of symbiotic N fixation obtained by monitoring net N mineralization in soil during crop growth with estimates obtained by a standard method (e.g. ^{15}N enrichment). A simple method for estimating the total amount of symbiotically fixed N in a legume crop would be a valuable research tool.

CHAPTER V

THE PHOSPHORUS NUTRITION OF TWO GRAIN LEGUMES AS AFFECTED BY THE MODE OF NITROGEN NUTRITION

II. EXTERNAL AND INTERNAL PHOSPHORUS REQUIREMENTS FOR GROWTH

INTRODUCTION

The physiology and morphology of a symbiotic, N-fixing legume differ markedly from its N-fertilized counterpart. Effective N fixation by the legume-Rhizobium symbiosis necessitates root nodulation and the biosynthesis of specific enzymes and proteins not found in the nonnodulated plant. Consequently, N-fixing legumes appear to have greater requirements for the mineral nutrients Mo (Anderson and Spencer, 1950), Co (Reisenauer, 1960), and Ca (Loneragan, 1959). Inadequate supplies of these nutrients induce N deficiency symptoms in plants dependent upon symbiotic N fixation indicating that they are required for at least one physiological process necessary to establish or maintain the symbiosis (Anderson, 1956; Reisenauer, 1960; Lowther and Loneragan, 1970). There are no definitive studies which indicate that the requirements for other essential nutrients differ. Although deficiencies of P, K, and S have been shown to limit N fixation, it is not clear if they are limiting growth of the host plant or directly inhibiting nodulation or N fixation (Munns, 1977).

The most obvious difference between the N-fixing and N-supplied legume is in their root morphology. Nodule mass comprises a large proportion of the total dry weight of the underground portion of plants dependent on symbiotic N fixation. In sand culture using a -N nutrient solution, Summerfield et al (1977) found nodules to include

32% of the dry weight of the entire root system of cowpea at first flowering. A similar value of 38% was reported for soybeans in Chapter III. Other studies have shown that there is a negative correlation between lateral root formation and nodule number in some legume species (Nutman, 1948; Dart and Pate, 1959). The data presented in Chapter III demonstrated that soybean plants provided with adequate quantities of combined N had larger and more extensive root systems than their N-fixing counterparts. In the same study it was also shown that when moderately stressed for P, the N-supplied soybean plants had a larger root mass than the N-fixing plants in both absolute and relative terms.

Nutrient uptake from soil by plants depends on: (1) total root surface area, (2) nutrient uptake per unit root surface, and (3) the physio-chemical characteristics of the soil. The more extensive root system of an N-supplied legume would be expected to increase the capacity of the roots to absorb mineral nutrients from soil above that of an N-fixing plant unless there is a compensatory increased nutrient uptake per unit root surface area of N-fixing plants. The experiment in this study was conducted to compare the critical external and internal P requirements as affected by the predominant mode of N nutrition during crop growth for both soybean and cowpea.

MATERIALS AND METHODS

The experimental site, soil characteristics, basal fertilizer applications, cultural practices, and experimental design have been previously described in detail (Chapter IV). Briefly, phosphorus

fertilizer application rates were chosen to establish the concentration of P in solutions equilibrated with soil at 0.0015, 0.011, 0.024, 0.070, 0.170, and 0.500 ppm P as determined by the method of Fox and Kamprath (1970). These 6 treatment levels required P application rates of 0, 400, 620, 960, 1360, and 1880 kg/ha. The P fertilizer was applied one year before this experiment was planted and during the intervening period a uniformity trial with corn was conducted to assess soil N availability (Chapter IV). Soil N availability was modified by incorporating bagasse into the entire field and establishing two N-level subplots within each P mainplot. One subplot received no N fertilizer (N-deficient) and to the other subplot N fertilizer was applied at a level estimated to supply sufficient N to both counteract the immobilization of soil N by the bagasse and meet the total crop N requirement (N-luxuriant). Both soybean and cowpea were inoculated with superior strains of Rhizobium in sufficient numbers to assure optimum nodulation. Three 5 meter rows of both soybean and cowpea were planted in each subplot on June 6, 1978. Soybean rows were spaced 0.75 m apart and intrarow spacing was 3.0 cm (333,000 plants/ha). Cowpea rows were spaced 1 m apart and intrarow spacing was 7.5 cm (133,000 plants/ha). The soybean variety used ("Clark 63") was a determinant cultivar of medium stature. The cowpea cultivar used (TVu 745-P3) was an indeterminant, viny entry from the 1976 Internation Cowpea Disease Nursery from the International Institute of Tropical Agriculture, Ibadan, Nigeria and identified as having a high grain yield potential by Kang and Fox (in press). Overhead irrigation was employed when necessary to maintain adequate soil moisture levels during crop growth.

Four soybean plants and 3 cowpea plants were randomly sampled from the middle border row within each subplot at 21, 32, 46, and 60 days after emergence. Plant tops were oven dried at 55°C and weighed. Relative growth rates were calculated for the 20 to 32, 32 to 46, 46 to 60 day, and the 60 day to harvest growth periods using the formula:

$$RGR = \ln W_2 - \ln W_1 / t_2 - t_1$$

where W_2 and W_1 are the top dry weights at time t_2 and t_1 which are the days from emergence for the later and earlier sampling times, respectively. The first fully expanded leaf was sampled from 20 plants in the harvest row of each subplot 42 days after emergence, dried at 55°C, ground, and 0.5 g digested by a modified Kjeldahl procedure. Nitrogen concentration in these index tissue samples was determined colorimetrically (Mitchell, 1972) and the P concentration measured from the same digest using the ascorbic acid phosphomolybdate-blue method developed by Murphy and Riley (1962) which was shown to be effective for plant tissue P analysis by Throneberry (1974). Fine roots < 2mm diameter were collected from three soybean and three cowpea plants randomly sampled from each subplot 50 days after emergence. After washing and staining as described by Phillips and Hayman (1970), mycorrhizal infection was rated on a scale of 0 to 10 by examination with a dissecting microscope.

Soybean plants from the N-deficient subplots matured 3 days earlier than those in the N-luxuriant subplots. Consequently the soybeans in the N-deficient subplots were harvested 81 days after emergence and the N-luxuriant soybeans 3 days later. At harvest soybean plants from 2 meters of the middle row in each subplot were cut at the

soil surface and the number of plants counted. The plants were dried at 55°C, seed and haulm yields determined, and subsamples ground for N and P analysis as previously described.

Cowpea was harvested from both the N-deficient and N-luxuriant subplots 90 days after emergence. Plants from 2 meters of the middle row in each subplot were cut at the soil surface, counted, fresh weight recorded, the plant material chopped, and two subsamples taken; one to determine moisture content and another for N and P analysis. At harvest there were mature dry pods, green pods, and flowers present on plants in all treatments.

Soil samples were collected from each subplot 28 days after emergence. The soil samples were air dried and screened through a 2 mm sieve. Soil P was measured by 3 methods: a modified Olsen's extraction (Banderis et al, 1976), Bray P1 extraction (Council on Soil Testing and Plant Analysis, 1974), and by equilibration for 6 days in 0.01M $CaCl_2$ (Fox and Kamprath, 1970). All yield data were correlated with the values obtained from these soil samples.

The critical external soil P requirement was based upon the 90% maximum yield level. Yield response curves for both soybean and cowpea best fit a Mitscherlich equation with 3 estimated parameters of the form:

$$Y = \theta_1 - \theta_2 e^{\theta_3 X}$$

where X is the soil P level, Y the predicted yield at any given X value, θ_1 the estimate of maximum yield at infinite soil P, θ_2 the estimate of the difference between the maximum yield and the estimated Y intercept and, θ_3 the estimate of the decreasing yield response to each added

increment of soil P. The parameters were estimated by the Marquardt algorithm for nonlinear equations using the Statistical Analysis System (Barr et al, 1976). The critical internal P concentration was estimated by linear regression of yield at harvest on the index tissue P concentration. The critical internal P concentration was considered to be the index tissue P concentration which corresponds to the 90% maximum yield on the regression line.

RESULTS

The Haiku clay soil at the experimental site is a Humoxic Tropohumult and has a relatively high P sorption capacity as shown in the P sorption isotherm (Fig. 10). The standard P requirement, defined by Fox and Searle (1978) as the phosphate required to adjust the soil to 0.2 ppm P in solution, is 720 µg P/g soil and is comparable to other soils with oxic mineralogy derived from weathered volcanic ash. The incorporation of bagasse into the soil did not affect the P sorption characteristics of the soil in either the N-deficient (-N) or N-luxuriant (+N) subplots. In each of the four lowest P treatments the available P, as measured by three soil test methods, was similar in the -N and +N subplots (Table 4). At the two highest P treatments the available P appeared to be higher in the -N subplots than in the +N subplots.

There was a significant yield response to each added increment of applied P fertilizer up to 620 kg P/ha for soybean from the -N subplots (Table 5). The yield response for soybean from +N subplots leveled off at application rates above 400 kg P/ha. At P application rates of 400,

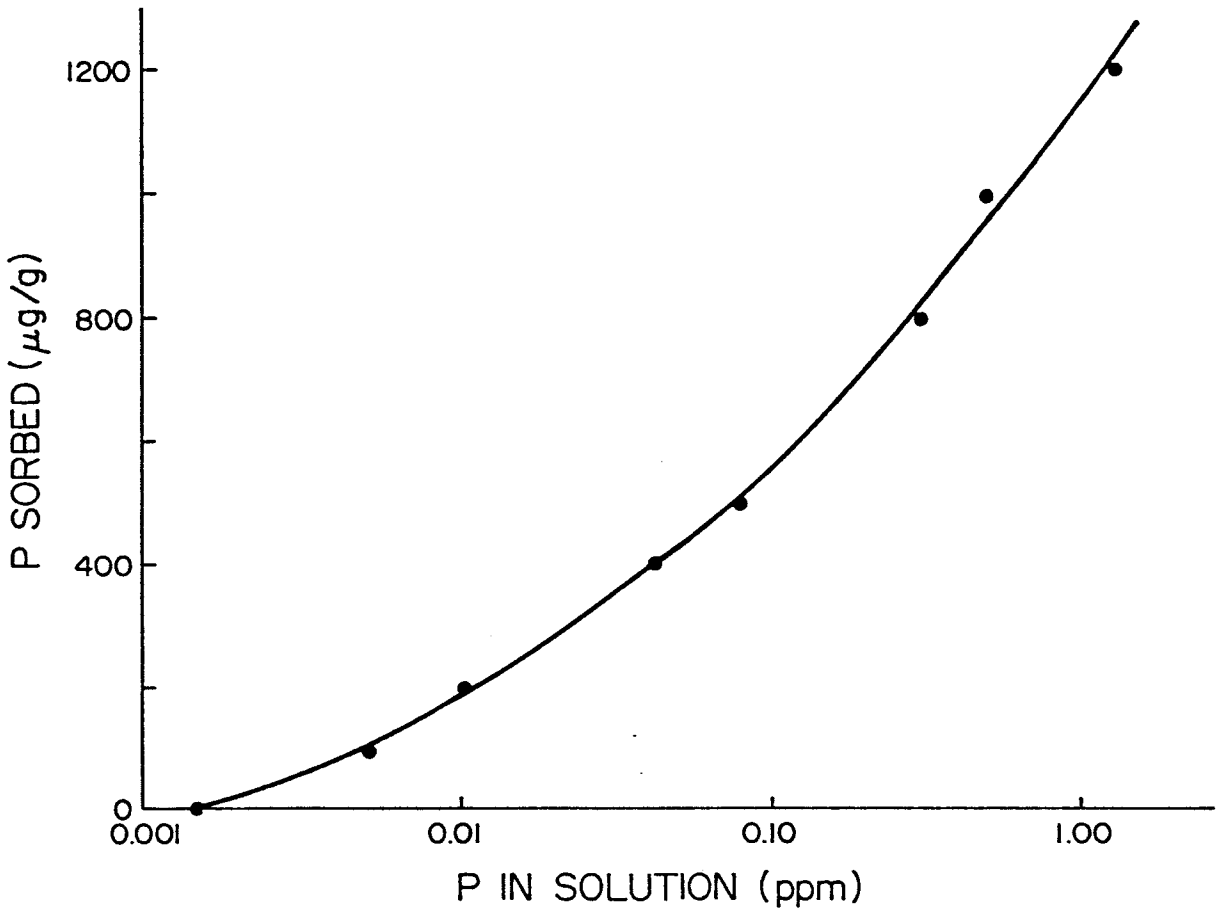


Figure 10. Phosphorus sorption isotherm for the Haikù clay surface soil (Humoxic Tropohumult)

Table 4. Phosphorus levels in the Haiku soil as affected by the rate of fertilizer P application*, extraction method, and N-deficient or N-luxuriant soil conditions.

P applied		Solution P**	Bray P	Olsen P
kg/ha		ppm		
0	-N	0.0015	0.4	2.5
	+N	0.0015	0.5	2.6
400	-N	0.004	1.6	8.0
	+N	0.004	1.7	7.7
620	-N	0.007	3.8	14.1
	+N	0.007	3.5	14.7
960	-N	0.012	6.8	21.9
	+N	0.012	6.1	21.3
1360	-N	0.018	11.4	33.3
	+N	0.016	9.6	31.6
1880	-N	0.074	35.8	74.2
	+N	0.034	21.9	53.4

*Phosphorus fertilizer applied one year before soil samples collected for analysis.

**Solution P is the concentration of P in a 0.01 M $CaCl_2$ solution equilibrated for 6 days with soil (Fox and Kamprath, 1970).

Table 5. The yield, relative yield, index tissue[+] P content, and P content of harvested soybean seed and cowpea dry matter as affected by the mode of N nutrition and soil P level.

fertilizer P applied	soybean				cowpea			
	seed yield	relative yield	index tissue P content	seed P content	dry matter yield	relative yield	index tissue P content	dry matter P content
kg/ha	kg/ha	% maximum yield	%	%	kg/ha	% maximum yield	%	%
0 -N	1030	28[++]	0.22	0.39	4348	72[++]	0.21	0.23
+N	934	19	0.21	0.35	3727	54	0.19	0.22
400 -N	2699	74	0.37	0.54	5727	94	0.47	0.34
+N	4207	87	0.49	0.61	6592	96	0.50	0.32
620 -N	3129	86	0.43	0.55	5907	97	0.49	0.35
+N	4468	92	0.49	0.64	6812	99	0.52	0.34
960 -N	3303	90	0.44	0.55	6025	99	0.49	0.33
+N	4586	95	0.53	0.65	6797	99	0.52	0.34
1360 -N	3490	96	0.48	0.57	5853	96	0.50	0.34
+N	4836	100	0.52	0.63	6848	100	0.50	0.32
1880 -N	3638	100	0.50	0.60	6075	100	0.50	0.35
+N	4695	97	0.54	0.65	6830	100	0.52	0.33
$LSD_{.05}$(N)*	210		0.03	0.04	450		n.s.	n.s.
$LSD_{.05}$(N x P)**	305		0.04	0.04	750		0.04	0.05

[+] Index tissue for both legumes was the first fully-expanded leaf sampled 6 weeks after emergence.
[++] Relative yields were determined separately for +N and -N phosphorus treatments.
* Used for comparisons between different N levels at the same P level.
** Used for comparisons between different N levels at different P levels or to compare the same N levels at different P levels.

620, and 960 kg P/ha the relative yield for soybean from the +N subplots was higher than in -N subplots at comparable P levels (Table 5). When no P fertilizer was applied, soybean seed yields from -N and +N treatments were equivalent. In all plots which received P fertilizer applications, the yield of soybean was significantly higher in the +N treatments than in the -N treatments (Table 5). The maximum yield of N-fixing soybean was approximately 75% that of N-supplied plants.

In plots to which no P fertilizer was applied there was a significantly larger yield in the -N treatment than in the P_0+N treatment (Table 5), Within the same N treatment, cowpea yields did not differ significantly at any level of applied P above P_0. Cowpea dry matter yield was also significantly higher in the +N subplots at all levels of applied P fertilizer. The maximum yield of N-fixing cowpea was 87% that of N-supplied plants. The relative difference between the maximum yield of N-fixing and N-supplied cowpea was less than for soybean.

In soybean, both the index tissue P concentration and the seed P concentration differed significantly between N treatments at any given level of applied P fertilizer (Table 5). However, in plots to which no P was applied, the index tissue P concentrations were similar. Regression of soybean seed yield on index tissue P concentration indicated that the relationships between these two parameters were significantly different in the N-deficient and N-luxuriant treatments (Fig. 11). The regression equations derived for the -N and +N treatments had significantly different mean square deviations for regression, slopes, and elevations. However, the critical index tissue P concentrations corresponding to 90% of their respective maximum yields were nearly

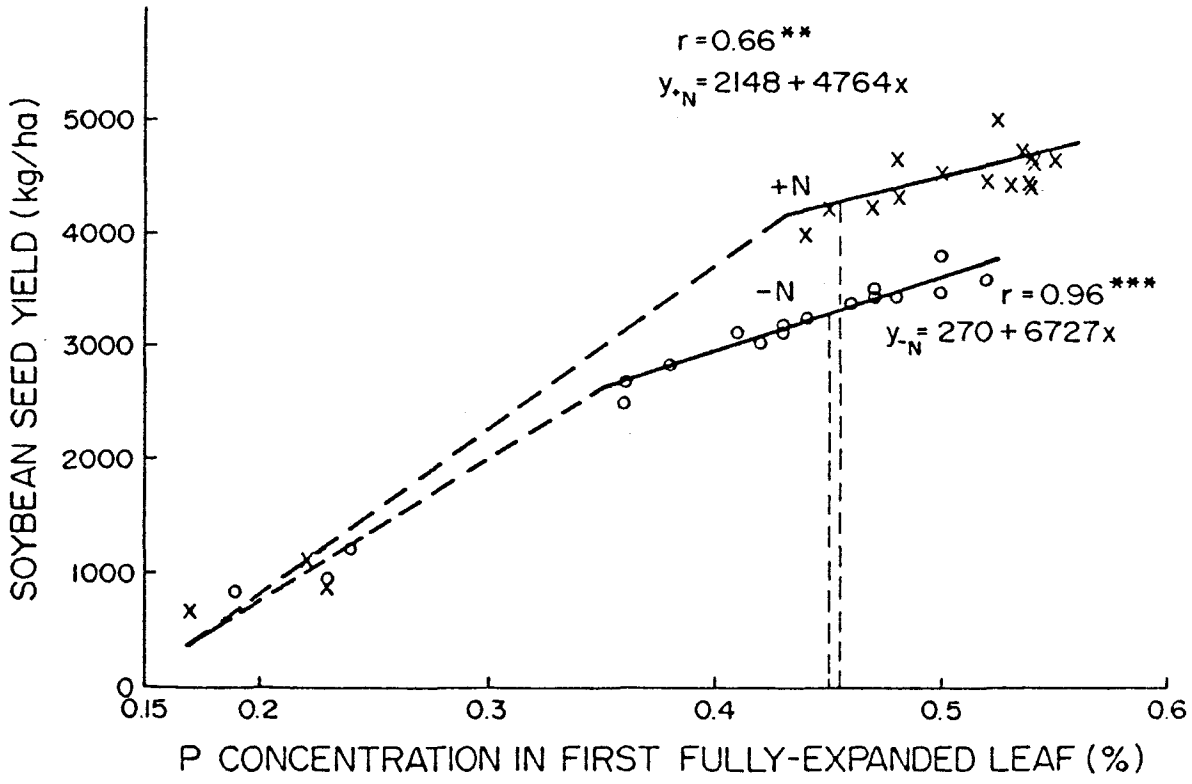


Figure 11. Relationship between soybean seed yield and P concentration in the index tissue (first fully-expanded leaf sampled 42 days after emergence). Dashed lines represent the critical internal P % required for 90% maximum yield of N-fixing (-N) and N-supplied (+N) plants.

identical, both approximately 0.45%. For cowpea the soil N level had no significant effect on the index tissue P concentration or the harvested dry matter P concentration at any given P level (Table 5). There was no significant correlation between the dry matter yields and index tissue P concentrations for -N or +N treatments and consequently a critical index tissue P concentration could not be obtained with this data. However it is obvious the critical P concentration in the first fully-expanded leaf of cowpea was greater than 0.21% and less than 0.5% (Table 5).

To simplify the discussion of the P response of soybean and cowpea during crop growth, 3 categories of soil P availability were made from the 6 P treatment levels: (1) "low P" which corresponds to the 0 P applied treatment, (2) "medium P" which corresponds to the 400 kg P/ha treatments and, (3) "high P" which is a grouping of the data from the 3 highest P application treatments. These P treatment classifications were chosen because there were large differences in the early growth and relative growth rates among these 3 treatment categories for both soybean and cowpea. Data from the 3 highest P applied treatments were combined because there were no significant differences in early growth, relative growth rates, or yield among these treatments for both soybean and cowpea.

The early growth response of soybean to P was not as great as that of cowpea. Top weights of soybean plants 21 days after emergence from the high P, +N treatments were 2.0 and 1.3 times that of plants from the low and medium P, +N treatments, respectively (Fig. 12). At harvest the yield of soybean from the high P, +N treatments increased to 5

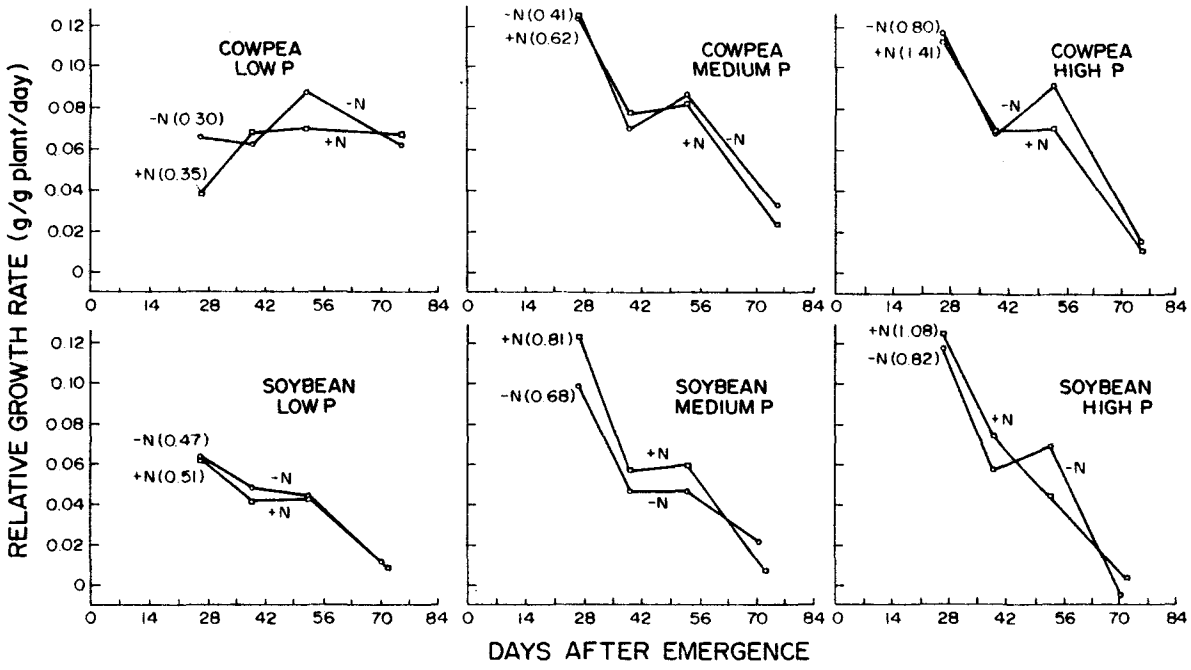


Figure 12. Relative growth rates of N-fixing (-N) and N-supplied (+N) soybean and cowpea plants during crop growth. Numbers in parentheses represent the top dry weight of plants sampled at 21 days after emergence.

times that obtained from the low P, +N treatment while there was little change in the relative yield obtained from the medium P, +N treatment at 21 days and at harvest. The early growth response of soybean plants from -N treatments to P was similar to that in the +N treatments.

Although there were no significant differences in the dry matter yield of cowpea at any level of applied P within the same N treatment at harvest, there was a dramatic response to applied P during early growth. Twenty-one days after emergence the top weight of +N cowpea plants from the high P treatments was 4 and 2 times that of the +N plants from the low and medium P treatments, respectively (Fig. 12). Unlike soybean, at harvest these differences had narrowed considerably. The early growth response of -N cowpea plants to P was relatively less than that of the +N plants although the trends were similar at 21 days after emergence.

There were distinct differences between the relative growth rate (RGR) patterns of soybean and cowpea during crop growth (Fig. 12) In contrast to soybean, at low P cowpea was able to maintain a high RGR during the later stages of growth. Also, under optimal growth conditions (high P, +N) cowpea maintained a constant and generally higher RGR during the 32 to 60 day growth interval while the soybean RGR had decreased substantially during this period. This is indicative of the more indeterminant nature of the cowpea cultivar used. Finally, -N soybean plants from the low and medium P treatments did not have a RGR peak during the 46 6o 60 day interval as did cowpea. There were also notable differences between the RGR patterns of N-fixing and N-supplied plants. At all P levels the N-fixing cowpea plants had a

higher RGR during the 46 to 60 day growth period than during the preceeding 32 to 46 day period while the N-supplied plants maintained a constant RGR during this time (Fig. 12). Nitrogen-fixing soybean plants grown at high P levels also had a RGR peak during the 46 to 60 day period while the RGR of N-supplied plants declined during the 32 to 60 day period.

The relationship between the yield of both soybean and cowpea and the soil P as quantified by (1) P applied, (2) soil equilibration in 0.01M $CaCl_2$, (3) Bray P1 extraction and, (4) Olsen's extraction is presented in Figures 13 to 16. The correlation between yield and each of the four soil P parameters was highly significant. Using the Mitscherlich equations that best fit the data, the maximum yield and the critical external P requirements for both soybean and cowpea are presented in Table 6. Three important points should be made. First, the critical external P requirement for soybean is affected by soil N availability. Under N-luxuriant soil conditions the external P requirement was approximately 60% lower than for soybean plants grown on N-deficient soil as estimated by all four methods of soil P evaluation. Second, soil N availability had no effect on the external P requirements of cowpea. Third, the cowpea cultivar used appears to be more tolerant of low soil P levels than does soybean regardless of soil N status.

Assessment of the mycorrhizal infection of fine roots of both soybean and cowpea collected from plants 50 days after emergence indicated that the roots of both legumes were heavily mycorrhizal in all of the P and N treatments. There were no statistically significant

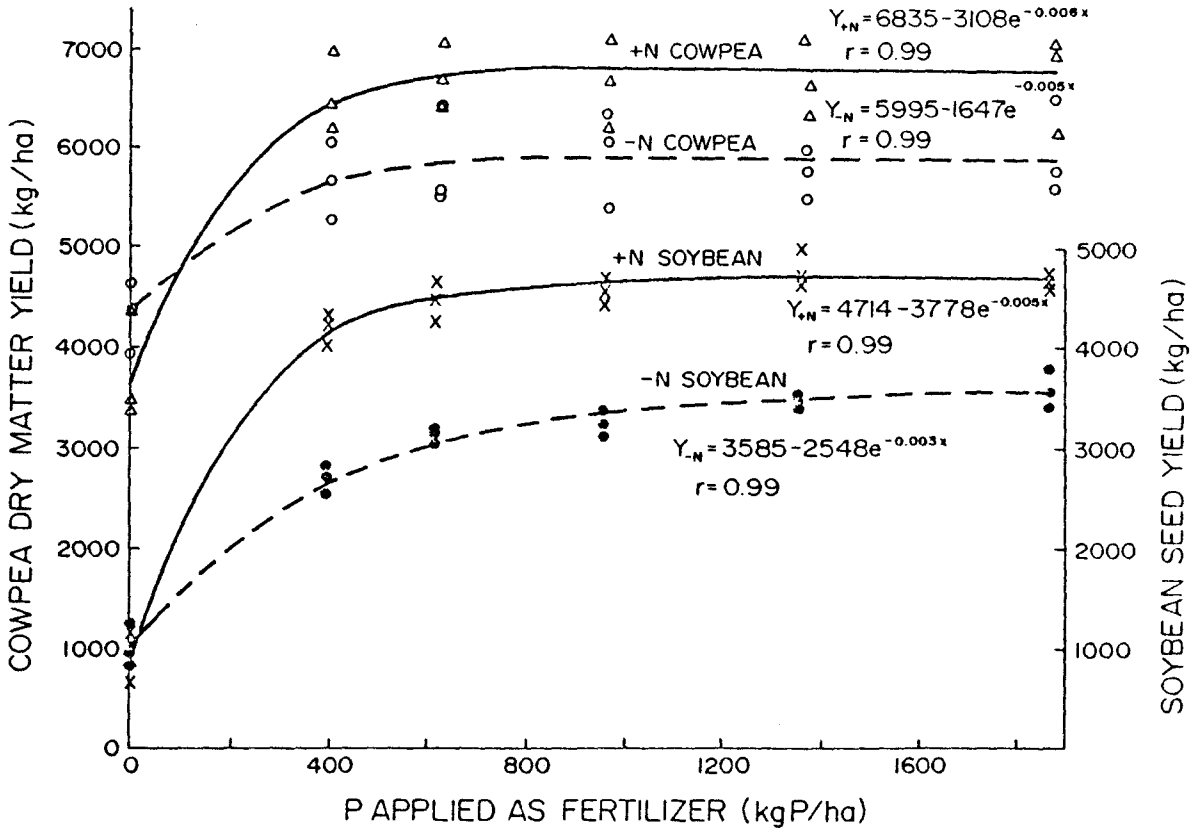


Figure 13. Yield response of N-fixing and N-supplied soybean and cowpea to soil P as quantified by P applied as fertilizer.

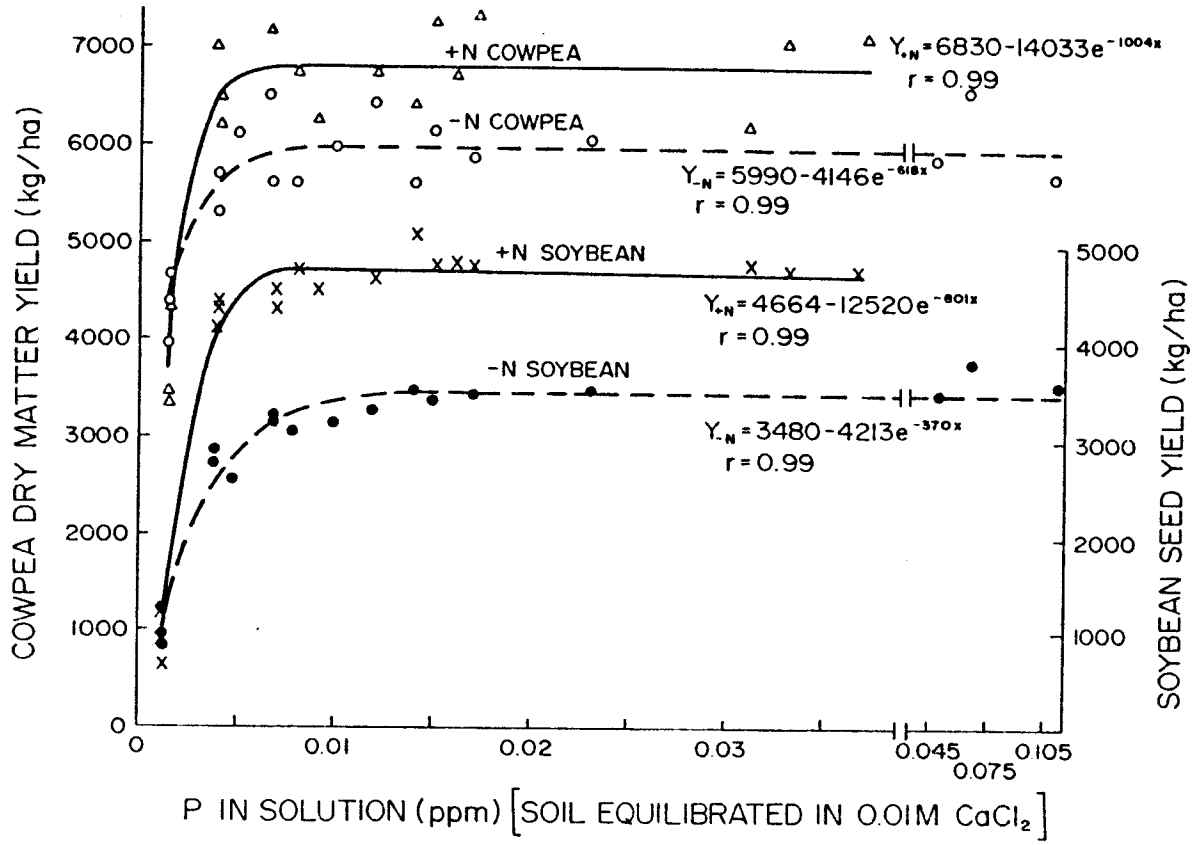


Figure 14. Yield response of N-fixing and N-supplied soybean and cowpea to soil P as quantified by the concentration of P in a 0.01 M $CaCl_2$ solution equilibrated with soil for six days.

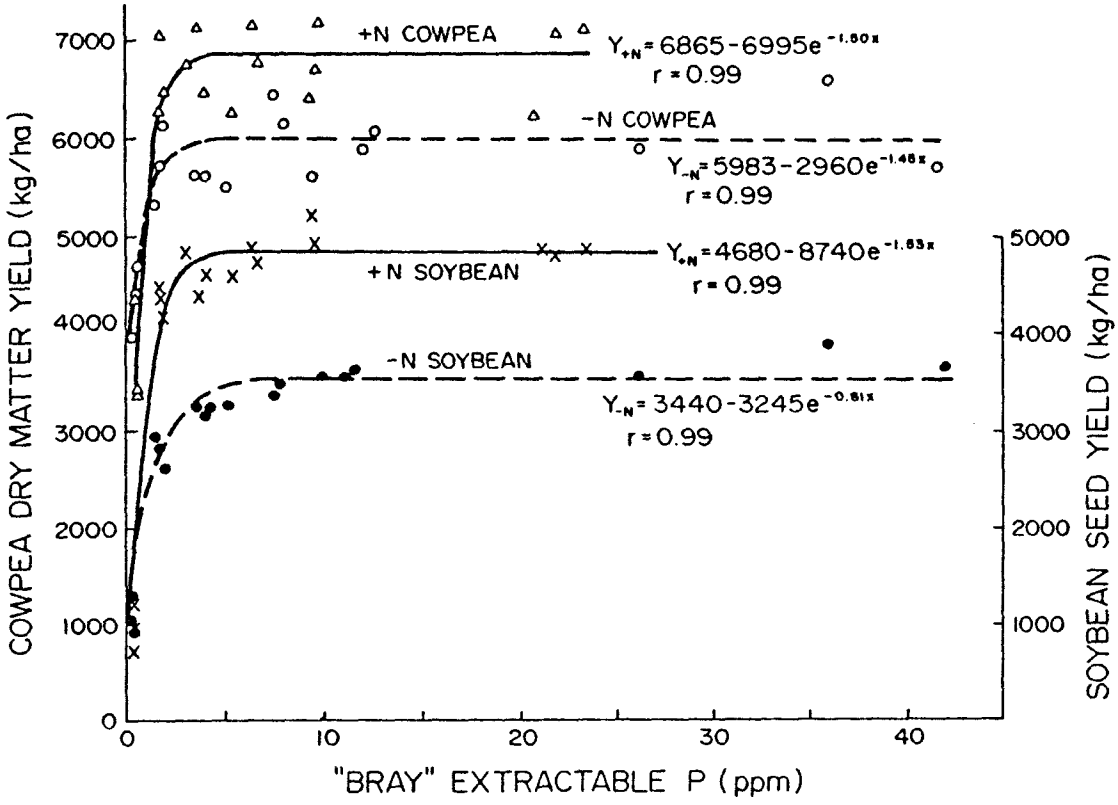


Figure 15. Yield response of N-fixing and N-supplied soybean and cowpea to soil P as quantified by "Bray" extractable P.

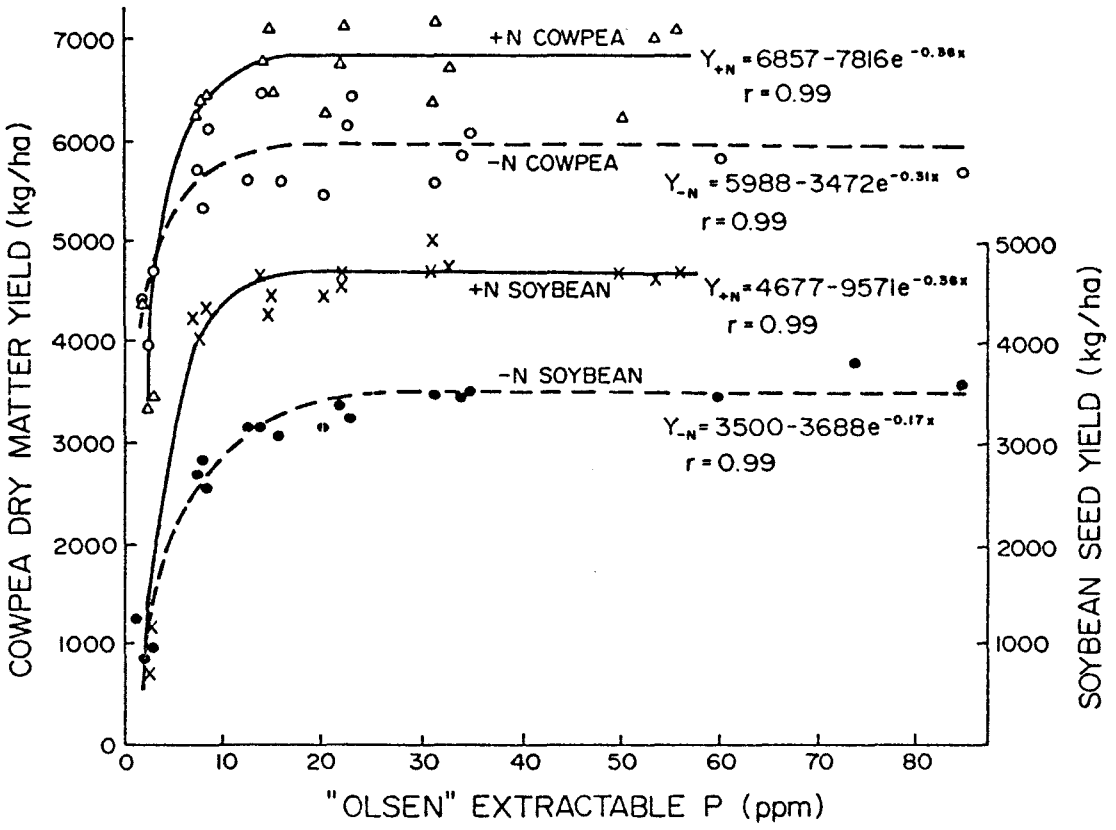


Figure 16. Yield response of N-fixing and N-supplied soybean and cowpea to soil P as quantified by "Olsen's" extractable P.

Table 6. The effect of Soil N availability upon the external P requirement of soybean and cowpea as estimated by four methods.

Soil N status	P applied		Solution P*		"Bray" P		"Olsen" P	
	Estimated maximum yield	P required for 90% max. yield	Estimated maximum yield	P required for 90% max. yield	Estimated maximum yield	P required for 90% max. yield	Estimated maximum yield	P required for 90% max. yield
	kg/ha		kg/ha	ppm	kg/ha	ppm	kg/ha	ppm
Soybean								
-N	3590	750	3480	0.007	3440	2.8	3500	13.5
+N	4710	430	4660	0.004	4680	1.9	4680	8.3
Cowpea								
-N	6000	220	5990	0.003	5980	1.1	5990	5.7
+N	6830	230	6830	0.003	6860	1.5	6860	6.7

* Solution P is the concentration of P in a 0.01M $CaCl_2$ solution equilibrated for 6 days with soil (Fox and Kamprath, 1970).

differences among any of the treatments for both soybean and cowpea. The intensity of infection appeared to be similar in the roots of both legumes.

DISCUSSION

Valid comparison of the mineral nutrient requirements of an N-fixing and N-supplied legume under field conditions requires that 3 conditions be met. First, soil N availability must be controlled such that the N-fixing legume is primarily dependent upon symbiotic N fixation to fulfill its N requirement for growth and the N-supplied legume is provided sufficient combined N to meet its N requirement. Second, highly effective strains of Rhizobium must be provided at planting to insure that the infection process and nodule development proceed at optimum rates unless specifically inhibited by the nutrient deficiency. Third, the soil modifications used to establish N-deficient and N-luxuriant soil conditions must not adversely affect the soil P availability to the plant. An attempt to verify that the first condition was satisfied was the topic of the first paper in this series (Chapter IV). The second condition was met by inoculating all seeds with highly effective rhizobial strains at rates approximately 40 times higher than that required for maximum soybean nodulation in soil (Chapter IV). The third condition has been discussed in this report and the data indicated that the soil P availability as estimated by three soil test methods was equivalent in the -N and +N subplots at each of the four lowest P treatment levels and that P availability

appeared to be higher in the -N subplots at the two highest P levels (Table 4). Increased microbial activity stimulated by high P and N-luxuriant soil conditions may have immobilized some of the labile soil P in these treatements. However, it is apparent that the lower soil P availability in the +N subplots at the two highest P levels was more than adequate for optimal growth and yield for both soybean and cowpea, and thus should not have affected the yield response to P.

With respect to P nutrition, the results from this experiment suggests there may be two classes of symbiotic, N-fixing legumes: those which are more sensitive to P stress when primarily dependent upon symbiotic N fixation, and those which have similar P requirements irrespective of the mode of N nutrition. In this study the soybean cultivar used typified the former class while the cowpea cultivar used represented the latter. The tolerance of cowpea to low soil P when effectively nodulated and grown on N-deficient soil could be characterized by: (1) a high RGR during the reproductive stages of growth, (2) tissue P concentrations which were similar to those from the N-supplied cowpea plants grown at comparable P levels, (3) an external P requirement which was not affected by soil N availability and, (4) a relatively small difference between the maximum yield obtained in the N-deficient and N-luxuriant treatments. The sensitivity of effectively nodulated soybean to low soil P when grown on N-deficient soil could be characterized by: (1) a RGR which declined progressively throughout the crop growth period, (2) lower index tissue and seed P concentrations than the N-supplied soybean plants grown at comparable P levels, (3) a higher external P requirement than the N-supplied soybean plants and, (4) a relatively larger difference between the

maximum yield obtained in the N-deficient and N-luxuriant treatments than for cowpea.

The fact that the cowpea cultivar used in this study was more tolerant of P stress than the soybean cultivar does not mean that all cowpea and soybean cultivars would behave similarly. It has been well documented that among genotypes within a plant species there are large differences in P feeding capacities (Lyness, 1936; Whiteaker et al, 1976; Nielsen and Barber, 1978). Nonetheless, a specific comparison of the two plants used in this experiment allows identification of the important features of tolerance or sensitivity to low soil P by the N-fixing grain legumes. It is noteworthy that Nanju (1973) reported a yield response to applied P fertilizer with soybean and not with cowpea on a Nigerian soil.

Analysis of the data obtained from this experiment does not provide answers as to why the soil N availability affected the internal tissue P levels and the external P requirement of soybean and not cowpea. We propose 3 hypotheses. One hypothesis is that symbiotic N fixation by soybean is more sensitive to P stress than in cowpea. Another hypothesis is that root development of soybean plants which are dependent upon symbiotically fixed N is slower than that of N-supplied soybean plants. Greenhouse pot experiments discussed in Chapter III showed that effectively nodulated soybean plants not provided combined N in the nutrient solution had less total root length than did plants provided combined N and that the relative difference in root development between N-fixing and N-supplied soybean plants was greatest at suboptimal P levels. Edwards and Barber (1976) reported that P uptake by soybean was related to total root length. No data are available on the root

development of cowpea as affected by the mode of N nutrition. A third hypothesis is that the mycorrhizal-cowpea symbiosis is much more efficient in the uptake of P under deficient soil P conditions irrespective of soil N availability and that at deficient soil N levels the efficiency of the mycorrhizal association in soybean is adversely affected. In this experiment the data collected on mycorrhizal root infection at 50 days after emergence did not support this hypothesis since the infection ratings did not differ among treatments or between the two legume species. It is possible that differences in mycorrhizal infection of roots existed during an earlier growth stage. Yost and Fox (in press) found that green pod weight of soybean and P uptake by cowpea were well correlated with an early mycorrhizal infection rating but not with a later rating. It is also possible that comparisons of the intensity of internal root mycorrhizal infection between plant species is not valid. These three hypotheses are not proposed independent of one another as a comprehensive understanding of the observed experimental results may involve all three.

In this experiment a "worst case" situation was established in the field such that soil N availability was either at deficient or luxuriant levels. In most arable soils the N availability would be intermediate between these two extremes. However, there are large potentially arable land areas in the humid and semi-arid tropics where light and water would not limit crop production (Sanchez, 1976; Uehara, 1977). Kellogg and Orvedal (1969) suggested that one of the major factors limiting utilization of tropical areas suitable for crop production is inadequate knowledge of how to manage the highly

weathered Oxisols and Ultisols in these regions. Nitrogen, lime, and phosphorus are the three major fertilizer inputs required to bring these soils into profitable agronomic production (Uehara, 1977). It is in these regions that symbiotic, N-fixing grain legume crops could provide high protein food without the use of petroleum-derived N fertilizers. The data presented in this report indicates that some N-fixing legumes can make respectable yields with little or no P fertilization while others might not. The cowpea cultivar used, grown without P or N fertilizer, yielded 72% of the maximum yield obtained at optimum P levels when effectively nodulated. The comparable relative yield for the soybean cultivar used was 28%. The data also indicated that the external P requirement for the soybean cultivar used in this experiment differs depending upon the soil N status. For the Ultisol on which this field experiment was conducted, an application of 750 kg P/ha was required to obtain a 90% maximum yield when soybeans were dependent upon symbiotic N fixation to meet their N requirement. This was 320 kg P/ha more than that required by N-supplied soybean plants to obtain a similar relative yield. Moreover, the maximum yield of N-supplied soybean was 30% greater than the maximum yield of N-fixing soybeans. Therefore to achieve a yield from N-supplied soybean plants equivalent in absolute terms to the 90% maximum yield of N-fixing plants required only 235 kg P/ha which is 515 kg P/ha less than that required by the N-fixing soybean plants. It was estimated that an application of 225 kg P/ha would be required for 90% maximum yield of cowpea and this was not influenced by soil N availability. We conclude that screening N-fixing grain legumes for tolerance to nutrient stress

under N-deficient soil conditions could become an important research objective.

CHAPTER VI

CONCLUSIONS

Soybean and cowpea were selected for study in these experiments because at present they represent two grain legumes with extremely different economic niches. Rachie (1977) states that of all tropical pulses, cowpeas are the most important source of human food in the lowland humid tropics. In the lowland humid tropics, cowpeas are mainly utilized in subsistence farming systems with little or no fertilizer input. By contrast, soybeans ranked first in value of all crops exported from the United States during the last 18 years (Hittle, 1975). Given their high export value, soybean farming in the United States is relatively intensive and requires inputs of fertilizer, weed and insect control, and the use of heavy machinery. Given their divergent economic and agronomic identities, it is not surprising that these two legumes were found to respond differently to P stress in a field experiment on a tropical soil.

The results from the experiments discussed in this dissertation can be summarized as follows: there was an inverse relationship between soybean root development and nodule mass; p stress affected nodule growth in soybean relatively more than either shoot or root growth; soybean was more sensitive to low soil P levels than was cowpea, especially when dependent upon symbiotically fixed N and grown on an N-deficient soil; and the tissue P concentrations and external P requirement of soybean were affected by soil N availability while cowpea tissue P concentrations and external P requirements were not affected by soil N

levels. These results indicate that cowpea would be a promising grain legume to use on potentially arable soils in the tropics where P is a major limiting factor to plant growth. Although there may be soybean genotypes which would be more tolerant to low soil P levels, these have not yet been identified.

It is their unique ability to convert symbiotically fixed atmospheric N_2 into protein that will make grain legumes an increasingly important human food source in the future, particularly on the potentially arable soils in developing countries of the tropics. The findings from these studies emphasize the importance of soil N availability in evaluating the critical nutrient requirements of some grain legumes. Fertilizer recommendations for soybean grown on N-deficient soils should be assessed with this in mind. The results also indicate that screening grain legumes for tolerance to low fertility levels should be conducted on N-deficient soils to insure that nutrient requirements are assessed for the symbiotic, N-fixing plant.

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